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(57) Abstract

The invention relates to genes isolated from *Arabidopsis* that code for proteins essential for seedling growth. The invention also includes the methods of using these proteins to discover new herbicides, based on the essentiality of the genes for normal growth and development. The invention can also be used in a screening assay to identify inhibitors that are potential herbicides. The invention is also applied to the development of herbicide tolerant plants, plant tissues, plant seeds, and plant cells.

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HERBICIDE TARGET GENE AND METHODS

The invention relates to genes isolated from *Arabidopsis* that code for proteins essential for seedling growth. The invention also includes the methods of using these proteins as an herbicide target, based on the essentiality of the gene for normal growth and development. The invention is also useful as a screening assay to identify inhibitors that are potential herbicides. The invention may also be applied to the development of herbicide tolerant plants, plant tissues, plant seeds, and plant cells.

The use of herbicides to control undesirable vegetation such as weeds in crop fields has become almost a universal practice. The herbicide market exceeds 15 billion dollars annually. Despite this extensive use, weed control remains a significant and costly problem for farmers.

Effective use of herbicides requires sound management. For instance, the time and method of application and stage of weed plant development are critical to getting good weed control with herbicides. Since various weed species are resistant to herbicides, the production of effective new herbicides becomes increasingly important. Novel herbicides can now be discovered using high-throughput screens that implement recombinant DNA technology. Metabolic enzymes found to be essential to plant growth and development can be recombinantly produced through standard molecular biological techniques and utilized as herbicide targets in screens for novel inhibitors of the enzyme activity. The novel inhibitors discovered through such screens may then be used as herbicides to control undesirable vegetation.

Herbicides that exhibit greater potency, broader weed spectrum, and more rapid degradation in soil can also, unfortunately, have greater crop phytotoxicity. One solution applied to this problem has been to develop crops that are resistant or tolerant to herbicides. Crop hybrids or varieties tolerant to the herbicides allow for the use of the herbicides to kill weeds without attendant risk of damage to the crop. Development of tolerance can allow application of a herbicide to a crop where its use was previously precluded or limited (*e.g.* to pre-emergence use) due to sensitivity of the crop to the herbicide. For example, U.S. Patent No. 4,761,373 to Anderson *et al.* is directed to plants resistant to various imidazolinone or sulfonamide herbicides. An altered acetohydroxyacid synthase (AHAS) enzyme confers the resistance. U.S. Patent No. 4,975,374 to Goodman *et al.* relates to plant cells and plants containing a gene encoding a mutant glutamine

synthetase (GS) resistant to inhibition by herbicides that were known to inhibit GS, *e.g.* phosphinothricin and methionine sulfoximine. U.S. Patent No. 5,013,659 to Bedbrook *et al.* is directed to plants expressing a mutant acetolactate synthase that renders the plants resistant to inhibition by sulfonylurea herbicides. U.S. Patent No. 5,162,602 to Somers *et al.* discloses plants tolerant to inhibition by cyclohexanedione and aryloxyphenoxypropanoic acid herbicides. The tolerance is conferred by an altered acetyl coenzyme A carboxylase (ACCase).

Notwithstanding the above described advancements, there remain persistent and ongoing problems with unwanted or detrimental vegetation growth (e.g. weeds). Furthermore, as the population continues to grow, there will be increasing food shortages. Therefore, there exists a long felt, yet unfulfilled need, to find new, effective, and economic herbicides.

For clarity, certain terms used in the specification are defined and presented as follows:

Chimeric: is used to indicate that a DNA sequence, such as a vector or a gene, is comprised of more than one DNA sequences of distinct origin which are fused together by recombinant DNA techniques resulting in a DNA sequence, which does not occur naturally, and which particularly does not occur in the plant to be transformed.

Co-factor: natural reactant, such as an organic molecule or a metal ion, required in an enzyme-catalyzed reaction. A co-factor is e.g. NAD(P), riboflavin (including FAD and FMN), folate, molybdopterin, thiamin, biotin, lipoic acid, pantothenic acid and coenzyme A, S-adenosylmethionine, pyridoxal phosphate, ubiquinone, menaquinone. Optionally, a co-factor can be regenerated and reused.

DNA shuffling: DNA shuffling is a method to rapidly, easily and efficiently introduce mutations or rearrangements, preferably randomly, in a DNA molecule or to generate exchanges of DNA sequences between two or more DNA molecules, preferably randomly. The DNA molecule resulting from DNA shuffling is a shuffled DNA molecule that is a non-naturally occurring DNA molecule derived from at least one template DNA molecule. The shuffled DNA encodes an enzyme modified with respect to the enzyme encoded by the template DNA, and preferably has an altered biological activity with respect to the enzyme encoded by the template DNA.

Enzyme activity: means herein the ability of an enzyme to catalyze the conversion of a substrate into a product. A substrate for the enzyme comprises the natural substrate of the enzyme but also comprises analogues of the natural substrate, which can also be

converted, by the enzyme into a product or into an analogue of a product. The activity of the enzyme is measured for example by determining the amount of product in the reaction after a certain period of time, or by determining the amount of substrate remaining in the reaction mixture after a certain period of time. The activity of the enzyme is also measured by determining the amount of an unused co-factor of the reaction remaining in the reaction mixture after a certain period of time or by determining the amount of used co-factor in the reaction mixture after a certain period of time. The activity of the enzyme is also measured by determining the amount of a donor of free energy or energy-rich molecule (e.g. ATP, phosphoenolpyruvate, acetyl phosphate or phosphocreatine) remaining in the reaction mixture after a certain period of time or by determining the amount of a used donor of free energy or energy-rich molecule (e.g. ADP, pyruvate, acetate or creatine) in the reaction mixture after a certain period of time.

Expression: refers to the transcription and/or translation of an endogenous gene or a transgene in plants. In the case of antisense constructs, for example, expression may refer to the transcription of the antisense DNA only.

Gene: refers to a coding sequence and associated regulatory sequences wherein the coding sequence is transcribed into RNA such as mRNA, rRNA, tRNA, snRNA, sense RNA or antisense RNA. Examples of regulatory sequences are promoter sequences, 5' and 3' untranslated sequences and termination sequences. Further elements that may be present are, for example, introns.

Herbicide: a chemical substance used to kill or suppress the growth of plants, plant cells, plant seeds, or plant tissues.

Heterologous DNA Sequence: a DNA sequence not naturally associated with a host cell into which it is introduced, including non-naturally occurring multiple copies of a naturally occurring DNA sequence; and genetic constructs wherein an otherwise homologous DNA sequence is operatively linked to a non-native sequence.

Homologous DNA Sequence: a DNA sequence naturally associated with a host cell into which it is introduced.

Inhibitor: a chemical substance that causes abnormal growth, e.g., by inactivating the enzymatic activity of a protein such as a biosynthetic enzyme, receptor, signal transduction protein, structural gene product, or transport protein that is essential to the growth or survival of the plant. In the context of the instant invention, an inhibitor is a chemical substance that alters the enzymatic activity encoded by the 245 gene, the 5283 gene, the 2490 gene, the 3963 gene or the 4036 gene from a plant. More generally, an

inhibitor causes abnormal growth of a host cell by interacting with the gene product encoded by the 245gene, the 5283 gene, the 2490 gene, the 3963 gene or the 4036 gene.

Isogenic: plants which are genetically identical, except that they may differ by the presence or absence of a heterologous DNA sequence.

Isolated: in the context of the present invention, an isolated DNA molecule or an isolated enzyme is a DNA molecule or enzyme that, by the hand of man, exists apart from its native environment and is therefore not a product of nature. An isolated DNA molecule or enzyme may exist in a purified form or may exist in a non-native environment such as, for example, in a transgenic host cell.

Marker gene: a gene encoding a selectable or screenable trait

Mature protein: protein which is normally targeted to a cellular organelle, such as a chloroplast, and from which the transit peptide has been removed.

Minimal Promoter: promoter elements, particularly a TATA element, that are inactive or that have greatly reduced promoter activity in the absence of upstream activation. In the presence of a suitable transcription factor, the minimal promoter functions to permit transcription.

Modified Enzyme Activity: enzyme activity different from that which naturally occurs in a plant (i.e. enzyme activity that occurs naturally in the absence of direct or indirect manipulation of such activity by man), which is tolerant to inhibitors that inhibit the naturally occurring enzyme activity.

Plant: refers to any plant, particularly to seed plants

Plant cell: structural and physiological unit of the plant, comprising a protoplast and a cell wall. The plant cell may be in form of an isolated single cell or a cultured cell, or as a part of higher organized unit such as, for example, a plant tissue, or a plant organ.

Plant material: refers to leaves, stems, roots, flowers or flower parts, fruits, pollen, pollen tubes, ovules, embryo sacs, egg cells, zygotes, embryos, seeds, cuttings, cell or tissue cultures, or any other part or product of a plant

Pre-protein: protein which is normally targeted to a cellular organelle, such as a chloroplast, and still comprising its transit peptide.

Recombinant DNA molecule: a combination of DNA sequences that are joined together using recombinant DNA technology

Selectable marker gene: a gene whose expression does not confer a selective advantage to a transformed cell, but whose expression makes the transformed cell phenotypically distinct from untransformed cells.

Significant Increase: an increase in enzymatic activity that is larger than the margin of error inherent in the measurement technique, preferably an increase by about 2-fold or greater of the activity of the wild-type enzyme in the presence of the inhibitor, more preferably an increase by about 5-fold or greater, and most preferably an increase by about 10-fold or greater.

Significantly less: means that the amount of a product of an enzymatic reaction is reduced by more than the margin of error inherent in the measurement technique, preferably a decrease by about 2-fold or greater of the activity of the wild-type enzyme in the absence of the inhibitor, more preferably an decrease by about 5-fold or greater, and most preferably an decrease by about 10-fold or greater.

In its broadest sense, the term "substantially similar", when used herein with respect to a nucleotide sequence, means a nucleotide sequence corresponding to a reference nucleotide sequence, wherein the corresponding sequence encodes a polypeptide having substantially the same structure and function as the polypeptide encoded by the reference nucleotide sequence, e.g. where only changes in amino acids not affecting the polypeptide function occur. Desirably the substantially similar nucleotide sequence encodes the polypeptide encoded by the reference nucleotide sequence. The term "substantially similar" is specifically intended to include nucleotide sequences wherein the sequence has been modified to optimize expression in particular cells. The percentage of identity between the substantially similar nucleotide sequence and the reference nucleotide sequence desirably is at least 65%, more desirably at least 75%, preferably at least 85%, more preferably at least 90%, still more preferably at least 95%, yet still more preferably at least 99%. Sequence comparisons are carried out using a Smith-Waterman sequence alignment algorithm (see e.g. Waterman, M.S. Introduction to Computational Biology: Maps, sequences and genomes. Chapman & Hall. London: 1995. ISBN 0-412-99391-0,). The localS program, version 1.16, is used with following parameters: match: 1, mismatch penalty: 0.33, open-gap penalty: 2, extended-gap penalty: 2. A nucleotide sequence "substantially similar" to reference nucleotide sequence hybridizes to the reference nucleotide sequence in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C with washing in 2X SSC, 0.1% SDS at 50°C, more desirably in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C with washing in 1X SSC, 0.1% SDS at 50°C, more desirably still in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C with washing in 0.5X SSC, 0.1% SDS at 50°C, preferably in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at

50°C, more preferably in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 65°C. As used herein the term "245 gene", "5283 gene", "2490 gene", "3963 gene" or "4036 gene" refers to a DNA molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9, respectively, or comprising a nucleotide sequence substantially similar to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:9, respectively. Homologs of the 245 gene , the 5283 gene, the 2490 gene, the 3963 gene or the 4036 gene include nucleotide sequences that encode an amino acid sequence that is at least 30% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10, respectively, as measured, using the parameters described below, wherein the amino acid sequence encoded by the homolog has the biological activity of the 245, 5283, 2490, 3963, or 4036 protein, respectively.

The term "substantially similar", when used herein with respect to a protein, means a protein corresponding to a reference protein, wherein the protein has substantially the same structure and function as the reference protein, e.g. where only changes in amino acids sequence not affecting the polypeptide function occur. When used for a protein or an amino acid sequence the percentage of identity between the substantially similar and the reference protein or amino acid sequence desirably is at least 65%, more desirably at least 75%, preferably at least 85%, more preferably at least 90%, still more preferably at least 95%, yet still more preferably at least 99%, using default BLAST analysis parameters. As used herein the term "245 protein", "5283 protein", "2490 protein", "3963 protein" or "4036 protein" refers to an amino acid sequence encoded by a DNA molecule comprising a nucleotide sequence substantially similar to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9, respectively. Homologs of the 245 protein , the 5283 protein, the 2490 protein, the 3963 protein or the 4036 protein are amino acid sequences that are at least 30% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10, respectively, as measured using the parameters described below, wherein the homologs have the biological activity of the 245, 5283, 2490, 3963, or 4036 protein, respectively.

One skilled in the art is also familiar with other analysis tools, such as GAP analysis, to determine the percentage of identity between the "substantially similar" and the reference nucleotide sequence, or protein or amino acid sequence. In the present invention, "substantially similar" is therefore also determined using default GAP analysis parameters with the University of Wisconsin GCG, SEQWEB application of GAP, based on the algorithm of Needleman and Wunsch (Needleman and Wunsch (1970) J Mol. Biol. 48: 443-

453).

Thus, in the context of the "245 gene" and using GAP analysis as described above, "substantially similar" refers to nucleotide sequences that encode a protein having at least 47% identity, more preferably at least 60% identity, still more preferably at least 75% identity, still more preferably at least 85% identity, still more preferably at least 95% identity, yet still more preferably at least 99% identity to SEQ ID NO:2.

In the context of the "5283 gene" and using GAP analysis as described above, "substantially similar" refers to nucleotide sequences that encode a protein having at least 74% identity, more preferably at least 80% identity, still more preferably at least 85% identity, still more preferably at least 90% identity, still more preferably at least 95% identity, yet still more preferably at least 99% identity to SEQ ID NO:4. Also, "substantially similar" preferably also refers to nucleotide sequences having at least 80% identity, more preferably at least 90% identity, still more preferably 95% identity, yet still more preferably at least 99% identity, to SEQ ID NO:3, wherein said nucleotide sequence comparisons are conducted using GAP analysis as described above.

In the context of the "2490 gene" and using GAP analysis as described above, "substantially similar" refers to nucleotide sequences that encode a protein having at least 82% identity, more preferably at least 85% identity, more preferably at least 90% identity, still more preferably at least 95% identity, yet still more preferably at least 99% identity to SEQ ID NO:6. Also, "substantially similar" preferably also refers to nucleotide sequences having at least 87% identity, more preferably at least 90% identity, still more preferably 95% identity, yet still more preferably at least 99% identity, to SEQ ID NO:5, wherein said nucleotide sequence comparisons are conducted using GAP analysis as described above.

In the context of the "3963 gene" and using GAP analysis as described above, "substantially similar" refers to nucleotide sequences that encode a protein having at least 40% identity, more preferably at least 60% identity, more preferably at least 80% identity, still more preferably at least 90% identity, still more preferably at least 95% identity, yet still more preferably at least 99% identity to SEQ ID NO:8. Also, "substantially similar" preferably also refers to nucleotide sequences having at least 49% identity, more preferably at least 60% identity, still more preferably 80% identity, more preferably at least 90% identity, more preferably at least 95% identity, yet still more preferably at least 99% identity, to SEQ ID NO:7, wherein said nucleotide sequence comparisons are conducted using GAP analysis as described above.

In the context of the "4036 gene" and using GAP analysis as described above, "substantially similar" refers to nucleotide sequences that encode a protein having at least 67% identity, more preferably at least 80% identity, more preferably at least 85% identity, still more preferably at least 90% identity, still more preferably at least 95% identity, yet still more preferably at least 99% identity to SEQ ID NO:10.

Further, using GAP analysis as described above, "homologs of the 245 gene" include nucleotide sequences that encode an amino acid sequence that has at least 24% identity to SEQ ID NO:2, more preferably at least 30% identity, still more preferably at least 40% identity, still more preferably at least 45% identity, yet still more preferably at least 55% identity, still more preferably at least 65% identity, yet still more preferably at least 75% identity to SEQ ID NO:2, wherein the amino acid sequence encoded by the homolog has the biological activity of the 245 protein.

Further, using GAP analysis as described above, "homologs of the 5283 gene" include nucleotide sequences that encode an amino acid sequence that has at least 23% identity to SEQ ID NO:4, more preferably at least 40% identity, still more preferably at least 50% identity, still more preferably at least 60% identity, yet still more preferably at least 74% identity to SEQ ID NO:4, wherein the amino acid sequence encoded by the homolog has the biological activity of the 5283 protein.

Further, using GAP analysis as described above, "homologs of the 2490 gene" include nucleotide sequences that encode an amino acid sequence that has at least 30% identity to SEQ ID NO:6, more preferably at least 30% identity, still more preferably at least 50% identity, still more preferably at least 60% identity, yet still more preferably at least 80% identity to SEQ ID NO:6, wherein the amino acid sequence encoded by the homolog has the biological activity of the 2490 protein.

Further, using GAP analysis as described above, "homologs of the 3963 gene" include nucleotide sequences that encode an amino acid sequence that has at least 34% identity to SEQ ID NO:8, more preferably at least 40% identity, still more preferably at least 50% identity, still more preferably at least 60% identity, yet still more preferably at least 75% identity to SEQ ID NO:8, wherein the amino acid sequence encoded by the homolog has the biological activity of the 3963 protein.

Further, using GAP analysis as described above, "homologs of the 4036 gene" include nucleotide sequences that encode an amino acid sequence that has at least 44% identity to SEQ ID NO:10, more preferably at least 50% identity, still more preferably at least 60% identity, yet still more preferably at least 75% identity to SEQ ID NO:10, wherein the

amino acid sequence encoded by the homolog has the biological activity of the 4036 protein.

When using GAP analysis as described above with respect to a protein or an amino acid sequence and in the context of the "245 gene", the percentage of identity between the "substantially similar" protein or amino acid sequence and the reference protein or amino acid sequence (in this case SEQ ID NO:2) is at least 47%, more preferably at least 60%, still more preferably at least 75%, still more preferably at least 85%, still more preferably at least 95%, yet still more preferably at least 99%. "Homologs of the 245 protein" include amino acid sequences that are at least 24% identical to SEQ ID NO:2, more preferably at least 30% identical, still more preferably at least 40% identical, still more preferably at least 45% identical, yet still more preferably at least 55% identical, still more preferably at least 65% identical, yet still more preferably at least 75% identical to SEQ ID NO:2, wherein homologs of the 245 protein have the biological activity of the 245 protein.

In the context of the "5283 gene" and using GAP analysis as described above, the percentage of identity between the substantially similar protein or amino acid sequence and the reference protein or amino acid sequence (in this case SEQ ID NO:4) is at least 74%, more preferably at least 80%, still more preferably at least 85%, still more preferably at least 90%, still more preferably at least 95%, yet still more preferably at least 99%. "Homologs of the 5283 protein" include amino acid sequences that at least 23% identity to SEQ ID NO:4, more preferably at least 40% identity, still more preferably at least 50% identity, still more preferably at least 60% identity, yet still more preferably at least 74% identity to SEQ ID NO:4, wherein homologs of the 5283 protein have the biological activity of the 5283 protein.

In the context of the "2490 gene" and using GAP analysis as described above, the percentage of identity between the substantially similar protein or amino acid sequence and the reference protein or amino acid sequence (in this case SEQ ID NO:6) is at least 82%, more preferably at least 85%, more preferably at least 90%, still more preferably at least 95%, yet still more preferably at least 99%. "Homologs of the 2490 protein" include amino acid sequences that have at least 30% identity to SEQ ID NO:6, more preferably at least 30% identity, still more preferably at least 50% identity, still more preferably at least 60% identity, yet still more preferably at least 80% identity to SEQ ID NO:6, wherein the homologs of the 2490 protein have the biological activity of the 2490 protein.

In the context of the "3963 gene" and using GAP analysis as described above, the percentage of identity between the substantially similar protein or amino acid sequence and the reference protein or amino acid sequence (in this case SEQ ID NO:8) is at least 40%,

more preferably at least 60%, more preferably at least 80%, still more preferably at least 90%, still more preferably at least 95%, yet still more preferably at least 99%. "Homologs of the 3963 protein" include amino acid sequences that has at least 34% identity to SEQ ID NO:8, more preferably at least 40% identity, still more preferably at least 50% identity, still more preferably at least 60% identity, yet still more preferably at least 75% identity to SEQ ID NO:8, wherein the homologs of the 3963 protein have the biological activity of the 3963 protein.

In the context of the "4036 gene" and using GAP analysis as described above, the percentage of identity between the substantially similar reference protein or amino acid sequence and the reference protein or amino acid sequence (in this case SEQ ID NO:10) is at least 67%, more preferably at least 80%, more preferably at least 85%, still more preferably at least 90%, still more preferably at least 95%, yet still more preferably at least 99%. "Homologs of the 4036 protein" include amino acid sequences that have at least 44% identity to SEQ ID NO:10, more preferably at least 50% identity, still more preferably at least 60% identity, yet still more preferably at least 75% identity to SEQ ID NO:10, wherein the homologs of the 4036 protein has the biological activity of the 4036 protein.

Substrate: a substrate is the molecule that an enzyme naturally recognizes and converts to a product in the biochemical pathway in which the enzyme naturally carries out its function, or is a modified version of the molecule, which is also recognized by the enzyme and is converted by the enzyme to a product in an enzymatic reaction similar to the naturally-occurring reaction.

Tolerance: the ability to continue essentially normal growth or function when exposed to an inhibitor or herbicide in an amount sufficient to suppress the normal growth or function of native, unmodified plants.

Transformation: a process for introducing heterologous DNA into a cell, tissue, or plant. Transformed cells, tissues, or plants are understood to encompass not only the end product of a transformation process, but also transgenic progeny thereof.

Transgenic: stably transformed with a recombinant DNA molecule that preferably comprises a suitable promoter operatively linked to a DNA sequence of interest.

SEQ ID NO:1 cDNA sequence for the Arabidopsis 245 gene

SEQ ID NO:2 amino acid sequence encoded by the Arabidopsis 245 DNA sequence shown in SEQ ID NO:1

SEQ ID NO:3 cDNA sequence for the Arabidopsis 5283 gene

SEQ ID NO:4 amino acid sequence encoded by the Arabidopsis 5283 DNA sequence shown in SEQ ID NO:3

SEQ ID NO:5 cDNA sequence for the Arabidopsis 2490 gene

SEQ ID NO:6 amino acid sequence encoded by the Arabidopsis 2490 DNA sequence shown in SEQ ID NO:5

SEQ ID NO:7 cDNA sequence for the Arabidopsis 3963 gene

SEQ ID NO:8 amino acid sequence encoded by the Arabidopsis 3963 DNA sequence shown in SEQ ID NO:7

SEQ ID NO:9 cDNA sequence for the Arabidopsis 4036 gene

SEQ ID NO:10 amino acid sequence encoded by the Arabidopsis 4036 DNA sequence shown in SEQ ID NO:9

SEQ ID NO:11 oligonucleotide SLP346for

SEQ ID NO:12 partial genomic sequence of the Arabidopsis 245 gene

SEQ ID NO:13 3'UTR from the cDNA sequence for the Arabidopsis 245 gene

SEQ ID NO:14 genomic sequence of the Arabidopsis 5283 gene

SEQ ID NO:15 oligonucleotide SLP328

SEQ ID NO:16 oligonucleotide LW60

SEQ ID NO:17 5'UTR from the cDNA sequence for the Arabidopsis 5283 gene

SEQ ID NO:18 3'UTR from the cDNA sequence for the Arabidopsis 5283 gene

SEQ ID NO:19 genomic sequence of the Arabidopsis 2490 gene

SEQ ID NO:20 5'UTR from the cDNA for the Arabidopsis 2490 gene

SEQ ID NO:21 3'UTR from the cDNA sequence for the Arabidopsis 2490 gene

SEQ ID NO:22 oligonucleotide SLP369

SEQ ID NO:23 oligonucleotide SLP370

SEQ ID NO:24 genomic sequence of the Arabidopsis 3963 gene

SEQ ID NO:25 oligonucleotide –21

SEQ ID NO:26 3'UTR from the cDNA sequence for the Arabidopsis 3963 gene

SEQ ID NO:27 genomic sequence of the Arabidopsis 4036 gene

SEQ ID NO:28 cDNA coding sequence for the Arabidopsis 4036 gene including variations between the cDNA and genomic sequence from cultivars Landsberg and Columbia

SEQ ID NO:29 amino acid sequence encoded by the Arabidopsis 4036 DNA sequence shown in SEQ ID NO:28

Encompassed by the invention is an isolated DNA molecule comprising a nucleotide sequence substantially similar to any one of the sequences selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9.. Preferred is the DNA molecule according to the invention, wherein the sequence encodes an amino acid sequence substantially similar to any one of the sequences selected from the group consisting of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10. Further preferred is DNA molecule according to the invention, wherein the sequence is any one of the sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9. Further preferred is the DNA molecule according to the invention, wherein the sequence encodes the amino acid sequence of any one of the sequences selected from the group consisting of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10. Further preferrred is a DNA molecule according to the invention, wherein said nucleotide sequence is a plant nucleotide sequence. More prefered is the DNA molecule according to the invention, wherein the plant *is Arabidopsis thaliana*. Further preferrred is a DNA molecule according to the invention, wherein the protein has any one of the activities selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 396 activity and 4036 activity. Further encompassed by the invention is an amino acid sequence comprising an amino acid sequence encoded by a nucleotide sequence substantially similar to any one of the sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9. Preferred is the amino acid sequence according to the invention comprising an amino acid sequence encoded by any one of the sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9. A further object of the invention is an amino acid sequence comprising an amino acid sequence substantially similar to any one of the sequences selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10. Preferred is the amino acid sequence according to the invention, wherein the sequence is any one of the sequences selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10. Further preferred is the amino acid sequence according to the invention, wherein the protein has any one of the activities selected from the group consisting of 245, 5283, 2490, 3963 and 4036 activity. Encompassed by the invention is an amino acid sequence comprising at least 20 consecutive amino acid residues of the amino acid sequence encoded by any one of the

sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9. Further encompassed is an amino acid sequence comprising at least 20 consecutive amino acid residues of the amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10. An object of the invention is an expression cassette comprising a promoter operatively linked to a DNA molecule according to the invention. Further encompassed by the invention is a recombinant vector comprising an expression cassette according to the invention, wherein said vector is capable of being stably transformed into a host cell. Further encompassed is a host cell comprising an expression cassette according to the invention, wherein said nucleotide sequence is expressible in said cell. Preferred is a host cell according to the invention, wherein said host cell is an eukaryotic cell. More preferred is a host cell according to the invention, wherein said host cell is selected from the group consisting of an insect cell, a yeast cell, and a plant cell. Also more preferred is a host cell according to the invention, wherein said host cell is a prokaryotic cell. Also more preferred is a host cell according to the invention, wherein said host cell is a bacterial cell. Encompassed is a plant or seed comprising a plant cell according to the invention. Preferred is a plant according to the invention, wherein said plant is tolerant to an inhibitor of any one of the activities selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity.

Further encompassed in the invention is a method comprising obtaining a host cell comprising a heterologous DNA molecule encoding a protein having 245, 5283, 2490, 3963, or 4036 activity; and expressing said protein in said host cell. Preferably the host cell is a bacterial cell, a yeast cell or an insect cell.

Further encompassed is a process for making nucleotides sequences encoding gene products having altered activity selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity comprising,

a) shuffling a nucleotide sequence of claim 1,

b) expressing the resulting shuffled nucleotide sequences and

c) selecting for altered activity selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity as compared to the activity selected

from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity of the gene product of said unmodified nucleotide sequence.

Preferred is a process according to the invention, wherein the nucleotide sequence is any one of the sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9. Encompassed by the invention is a shuffled DNA molecule obtainable by the process according to the invention. Encompassed by the invention is a shuffled DNA molecule produced by the process according to the invention. Further encompassed by the invention is a shuffled DNA molecule obtained by the according to the invention, wherein said shuffled DNA molecule encodes a gene product having enhanced tolerance to an inhibitor of any one of the activities selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity. A further object of the invention is an expression cassette comprising a promoter operatively linked to a nucleotide sequence according to the invention. Further encompased by the invention is a recombinant vector comprising an expression cassette according to the invention, wherein said vector is capable of being stably transformed into a host cell. A further object of the invention is a host cell comprising an expression cassette according the invention, wherein said nucleotide sequence is expressible in said cell. Preferred is a host cell according to the invention, wherein said host cell is an eukaryotic cell. Also preferred is a host cell according to the invention, wherein said host cell is selected from the group consisting of an insect cell, a yeast cell, and a plant cell. Also preferred is a host cell according to the invention, wherein said host cell is a prokaryotic cell. Also preferred is a host cell according to the invention, wherein said host cell is a bacterial cell. An object of the invention is a plant or seed comprising a plant cell according to the invention. Preferred is a plant according to the invention, wherein said plant is tolerant to an inhibitor selected from the group consisting of 245, 5283, 2490, 3963 and 4036 activity. Further encompassed is a method for selecting compounds that interact with the protein encoded by any one of the sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9 , comprising:

a) expressing a DNA molecule comprising any one of the sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9, respectively, or a sequence substantially similar to any one of the sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9 to generate the corresponding protein,

b) testing a compound suspected of having the ability to interact with the protein expressed in step (a), and

c) selecting compounds that interact with the protein in step (b).

A further object of the invention is a process of identifying an inhibitor of any one of the activities selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity comprising:

a) introducing a DNA molecule comprising a nucleotide sequence of any one of the sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9, respectively, and having any one of the activities selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity, or nucleotide sequences substantially similar thereto, or a homolog thereof, into a plant cell, such that said sequence is functionally expressible at levels that are higher than wild-type expression levels,

b) combining said plant cell with a compound to be tested for the ability to inhibit any one of the activities selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity under conditions conducive to such inhibition,

c) measuring plant cell growth under the conditions of step (b), and

d) comparing the growth of said plant cell with the growth of a plant cell having an unaltered activity selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity under identical conditions, and

e) selecting said compound that inhibits plant cell growth in step (d).

Encompassed by the invention is a compound having herbicidal activity identifiable according to the process according to the invention. Further encompassed is a process of identifying compounds having herbicidal activity comprising:

a) combining a protein according to the invention and a compound to be tested for the ability to interact with said protein, under conditions conducive to interaction,

b) selecting a compound identified in step (a) that is capable of interacting with said protein,

c) applying identified compound in step (b) to a plant to test for herbicidal activity, and

d) selecting compounds having herbicidal activity.

Further encompassed is a compound having herbicidal activity identifiable according to the process according to the invention. A further object of the invention is a method for suppressing the growth of a plant comprising, applying to said plant a compound that inhibits the activity of the amino acid sequence according to the invention in an amount sufficient to suppress the growth of said plant.
Preferred is the method according to the invention, wherein the compound is a compound having herbicidal activity identifiable according to the process according to the invention.
Encompassed is a method of improving crops comprising, applying to a herbicide tolerant plant or seed according to the invention, a compound having herbicidal activity identifiable according to a process according to the invention, in an amount that inhibits the growth of undesired vegetation without significantly suppressing the growth of the herbicide tolerant plant or seed. An object of the invention is a DNA molecule comprising a nucleotide sequence substantially similar to any one of the sequences selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29.
It is an object of the invention to provide an effective and beneficial method to identify novel herbicides. A feature of the invention is the identification of a gene in *Arabidopsis*, herein referred to as the 245 gene, which shows sequence similarity to peptide release factor 2 (Craigen et al. (1985) Proc. Natl. Acad. Sci, 82: 3616-3620; Craigen and Caskey (1987) Biochimie 69: 1031-1041; Ito et al. (1998) Proc. Natl. Acad. Sci., 95: 8165-8169). Another feature of the invention is the discovery that the 245 gene is essential for seedling growth and development. An advantage of the present invention is that the newly discovered essential gene containing a novel herbicidal mode of action enables one skilled in the art to easily and rapidly identify novel herbicides.

A further feature of the invention is the identification of a gene in *Arabidopsis*, herein referred to as the 5283 gene, which shows sequence similarity to the following: an uncharacterized gene from *Schizosaccharomyces pombe*; the *Saccharomyces cerevisiae* PRP31 gene that encodes a factor essential for pre-mRNA splicing (Weidenhammer et al. (1996) Nucleic Acids Res. 24: 1164-1170; Weidenhammer et al. (1997) Mol. Cell. Biol., 17:

3580-3585); the *Pisum sativum* SARBP-1 and SARBP-2 genes that encode Scaffold Attachment Region (SAR) DNA-binding proteins (Rzepecki et al. (1995) Acta Biochim. Pol., 42: 75-81); and the *Saccharomyces cerevisiae* SIK1 gene that encodes a protein that can suppress the growth inhibitory effects of IKB (Morin et al. (1995) Cell Growth & Differentiation, 6: 789-798). The SIK1 gene product is also referred to as Nop56, which is shown to be an essential nucleolar protein (Gautier et al. (1997) Mol. Cell. Biol. 17: 7088-7098). Another feature of the invention is the discovery that the 5283 gene is essential for seedling growth and development. An advantage of the present invention is that the newly discovered essential gene containing a novel herbicidal mode of action enables one skilled in the art to easily and rapidly identify novel herbicides.

A further feature of the invention is the identification of a gene in *Arabidopsis*, herein referred to as the 2490 gene, which encodes a protein with sequence similarity to a chloroplast envelope protein (Ko et al. (1995) The Journal of Biological Chem. 270: 28601-28608; Wu et al. (1994) The Journal of Biological Chem. 269: 32264-32271; Pang et al. (1997) The Journal of Biological Chem. 272: 25623-25627). Another feature of the invention is the discovery that the 2490 gene is essential for seedling growth and development. An advantage of the present invention is that the newly discovered essential gene containing a novel herbicidal mode of action enables one skilled in the art to easily and rapidly identify novel herbicides.

A further feature of the invention is the identification of a gene in *Arabidopsis*, herein referred to as the 3963 gene, which encodes a protein with sequence similarity to a number of DNA repair proteins, including Rad32p from *Schizosaccharomyces pombe* (Genbank accession numberQ09683); hMre11 from *Homo sapiens* (Genbank accession number U37359); and Mre11p from *Saccharomyces cerevisiae* (Genbank accession number U60829) (Johzuka and Ogawa (1995) Genetics, 139: 1521-1532; Paull and Gellert (1998) Molecular Cell, 1: 969-979). Another feature of the invention is the discovery that the 3963 gene is essential for seedling growth and development. An advantage of the present invention is that the newly discovered essential gene containing a novel herbicidal mode of action enables one skilled in the art to easily and rapidly identify novel herbicides.

A further feature of the invention is the identification of a gene in *Arabidopsis*, herein referred to as the 4036 gene, which encodes a protein with sequence similarity to 1-deoxy-D-xylulose 5-phosphate reductoisomerase from a number of organisms including *Synechocystis* sp. (SWISS-PROTQ55663), *Bacillus subtilis* (SWISS-PROT O31753), and *Escherichia coli* (SWISS-PROT P45568) (Takahashi et al. (1998) Proc. Natl. Acad. Sci. USA, 95: 9879-9884). An important and unexpected feature of the invention is the discovery that the 4036 gene is essential for seedling growth and development. An advantage of the present invention is that the newly discovered essential gene containing a novel herbicidal mode of action enables one skilled in the art to easily and rapidly identify novel herbicides.

One object of the present invention is to provide an essential gene in plants for assay development for inhibitory compounds with herbicidal activity. Genetic results show that when the 245 gene, the 5283 gene, the 2490 gene, the 3963 gene or the 4036 gene is mutated in *Arabidopsis*, the resulting phenotype is seedling lethal in the homozygous state. This suggests a critical role for the gene product encoded by the mutated gene.

Using T-DNA insertion mutagenesis, the inventors of the present invention have demonstrated that the activity encoded by the *Arabidopsis* 245 gene, the *Arabidopsis* 5283 gene, the *Arabidopsis* 2490 gene, the *Arabidopsis* 3963 gene or the *Arabidopsis* 4036 gene (herein referred to as 245, 5283, 2490, 3963 or 4036 activity) is essential in *Arabidopsis* seedlings. This implies that chemicals that inhibit the function of the protein in plants are likely to have detrimental effects on plants and are potentially good herbicide candidates. The present invention therefore provides methods of using a purified protein encoded by the gene sequences described below to identify inhibitors thereof, which can then be used as herbicides to suppress the growth of undesirable vegetation, e.g. in fields where crops are grown, particularly agronomically important crops such as maize and other cereal crops such as wheat, oats, rye, sorghum, rice, barley, millet, turf and forage grasses, and the like, as well as cotton, sugar cane, sugar beet, oilseed rape, and soybeans.

The present invention discloses a nucleotide sequence derived from *Arabidopsis*, designated the 245 gene. The nucleotide sequence of the cDNA clone is set forth in SEQ ID NO:1, and the corresponding amino acid sequence is set forth in SEQ ID NO:2. The nucleotide sequence of the partial genomic DNA sequence is set forth in ***SEQ ID NO:12***. The present invention also includes nucleotide sequences substantially similar to those set

forth in SEQ ID NO:1. The present invention also encompasses plant proteins whose amino acid sequence are substantially similar to the amino acid sequences set forth in SEQ ID NO:2. Such proteins can be used in a screening assay to identify inhibitors that are potential herbicides.

The present invention further discloses a nucleotide sequence derived from *Arabidopsis,* designated the 5283 gene. The nucleotide sequence of the cDNA clone is set forth in ***SEQ ID NO:3***, and the corresponding amino acid sequence is set forth in ***SEQ ID NO:4***. The nucleotide sequence of the genomic DNA sequence is set forth in ***SEQ ID NO:14***. The present invention also includes nucleotide sequences substantially similar to those set forth in ***SEQ ID NO:3***. The present invention also encompasses plant proteins whose amino acid sequence are substantially similar to the amino acid sequences set forth in ***SEQ ID NO:4***. Such proteins can be used in a screening assay to identify inhibitors that are potential herbicides.

The present invention further discloses a nucleotide sequence derived from *Arabidopsis,* designated the 2490 gene. The nucleotide sequence of the cDNA clone is set forth in ***SEQ ID NO:5***, and the corresponding amino acid sequence is set forth in ***SEQ ID NO:6***. The nucleotide sequence of the genomic DNA sequence is set forth in ***SEQ ID NO:19***. The present invention also includes nucleotide sequences substantially similar to those set forth in ***SEQ ID NO:5***. The present invention also encompasses plant proteins whose amino acid sequence are substantially similar to the amino acid sequences set forth in ***SEQ ID NO:6***. Such proteins can be used in a screening assay to identify inhibitors that are potential herbicides.

The present invention further discloses a nucleotide sequence derived from *Arabidopsis,* designated the 3963 gene. The nucleotide sequence of the cDNA clone is set forth in ***SEQ ID NO:7***, and the corresponding amino acid sequence is set forth in ***SEQ ID NO:8***. The nucleotide sequence of the genomic DNA sequence is set forth in ***SEQ ID NO:24***, which contains genomic DNA sequences from both the portion of the MDK4 clone annotated as MDK4.6 and added sequences on the 3' end based on the inventors' reported cDNA clone. The present invention also includes nucleotide sequences substantially similar to those set forth in in ***SEQ ID NO:7***. The present invention also encompasses plant proteins whose amino acid sequence are substantially similar to the amino acid sequences set forth in ***SEQ***

ID NO:8. Such proteins can be used in a screening assay to identify inhibitors that are potential herbicides.

The present invention further discloses a nucleotide sequence derived from *Arabidopsis*, designated the 4036 gene. The nucleotide sequence of the cDNA clone is set forth in ***SEQ ID NO:9***, and the corresponding amino acid sequence is set forth in ***SEQ ID NO:10***. The nucleotide sequence of the genomic DNA sequence is set forth in ***SEQ ID NO:27***. Thirteen nucleotide differences are observed by comparing the cDNA clone, derived from cv. Landsberg, and the genomic sequence, derived from cv. Columbia; and Table 1, below, further identifies these differences. ***SEQ ID NO:28*** is the same as ***SEQ ID NO:9***, but with these thirteen nucleotide differences. The corresponding amino acid sequence of ***SEQ ID NO:28*** is set forth ***in SEQ ID NO:29***. The present invention also includes nucleotide sequences substantially similar to those set forth in ***SEQ ID NO:9***. The present invention also encompasses plant proteins whose amino acid sequence are substantially similar to the amino acid sequences set forth ***in SEQ ID NO:10*** and ***SEQ ID NO:29***. Such proteins can be used in a screening assay to identify inhibitors that are potential herbicides.

In a preferred embodiment, the present invention relates to a method for identifying chemicals having the ability to inhibit 245, 5283, 2490, 3963 or 4036 activity in plants preferably comprising the steps of: a) obtaining transgenic plants, plant tissue, plant seeds or plant cells, preferably stably transformed, comprising a non-native nucleotide sequence encoding an enzyme having 245, 5283, 2490, 3963 or 4036 activity and capable of overexpressing an enzymatically active 245, 5283, 2490, 3963 or 4036 gene product (either full length or truncated but still active); b) applying a chemical to the transgenic plants, plant cells, tissues or parts and to the isogenic non-transfomed plants, plant cells, tissues or parts; c) determining the growth or viability of the transgenic and non-transformed plants, plant cells, tissues after application of the chemical; d) comparing the growth or viability of the transgenic and non-transformed plants, plant cells, tissues after application of the chemical; and e) selecting chemicals that suppress the viability or growth of the non-transgenic plants, plant cells, tissues or parts, without significantly suppressing the growth of the viability or growth of the isogenic transgenic plants, plant cells, tissues or parts. In a preferred embodiment, the enzyme having 245, 5283, 2490, 3963 or 4036 activity is encoded by a nucleotide sequence derived from a plant, preferably *Arabidopsis thaliana*, desirably identical or substantially similar to the nucleotide sequence set forth in SEQ ID

NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9, respectively. In another embodiment, the enzyme having 245, 5283, 2490, 3963 or 4036 activity is encoded by a nucleotide sequence capable of encoding the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10 respectively. In yet another embodiment, the enzyme having 245, 5283, 2490, 3963 or 4036 activity has an amino acid sequence identical or substantially similar to the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10 respectively.

The present invention further embodies plants, plant tissues, plant seeds, and plant cells that have modified 245, 5283, 2490, 3963 or 4036 activity and that are therefore tolerant to inhibition by a herbicide at levels normally inhibitory to naturally occurring 245, 5283, 2490, 3963 or 4036 activity. Herbicide tolerant plants encompassed by the invention include those that would otherwise be potential targets for normally inhibiting herbicides, particularly the agronomically important crops mentioned above. According to this embodiment, plants, plant tissue, plant seeds, or plant cells are transformed, preferably stably transformed, with a recombinant DNA molecule comprising a suitable promoter functional in plants operatively linked to a nucleotide coding sequence that encodes a modified 245, 5283, 2490, 3963 or 4036 gene that is tolerant to inhibition by a herbicide at a concentration that would normally inhibit the activity of wild-type, unmodified 245, 5283, 2490, 3963 or 4036 gene product. Modified 245, 5283, 2490, 3963 or 4036 activity may also be conferred upon a plant by increasing expression of wild-type herbicide-sensitive 245, 5283, 2490, 3963 or 4036 protein by providing multiple copies of wild-type 245, 5283, 2490, 3963 or 4036 genes to the plant or by overexpression of wild-type 245, 5283, 2490, 3963 or 4036 genes under control of a stronger-than-wild-type promoter. The transgenic plants, plant tissue, plant seeds, or plant cells thus created are then selected by conventional selection techniques, whereby herbicide tolerant lines are isolated, characterized, and developed. Alternately, random or site-specific mutagenesis may be used to generate herbicide tolerant lines.

Therefore, the present invention provides a plant, plant cell, plant seed, or plant tissue transformed with a DNA molecule comprising a nucleotide sequence isolated from a plant that encodes an enzyme having 245, 5283, 2490, 3963 or 4036 activity, wherein the DNA expresses the 245, 5283, 2490, 3963 or 4036 activity and wherein the DNA molecule confers upon the plant, plant cell, plant seed, or plant tissue tolerance to a herbicide in amounts that normally inhibits naturally occurring 245, 5283, 2490, 3963 or 4036 activity. According to one example of this embodiment, the enzyme having 245, 5283, 2490, 3963

or 4036 activity is encoded by a nucleotide sequence identical or substantially similar to the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9, respectively, or has an amino acid sequence identical or substantially similar to the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10, respectively.

The invention also provides a method for suppressing the growth of a plant comprising the step of applying to the plant a chemical that inhibits the naturally occurring 245, 5283, 2490, 3963 or 4036 activity in the plant. In a related aspect, the present invention is directed to a method for selectively suppressing the growth of undesired vegetation in a field containing a crop of planted crop seeds or plants, comprising the steps of: (a) optionally planting herbicide tolerant crops or crop seeds, which are plants or plant seeds that are tolerant to a herbicide that inhibits the naturally occurring 245, 5283, 2490, 3963 or 4036 activity; and (b) applying to the herbicide tolerant crops or crop seeds and the undesired vegetation in the field a herbicide in amounts that inhibit naturally occurring 245, 5283, 2490, 3963 or 4036 activity, wherein the herbicide suppresses the growth of the weeds without significantly suppressing the growth of the crops.

Other objects and advantages of the present invention will become apparent to those skilled in the art from a study of the following description of the invention and non-limiting examples.

As shown in the examples below, the identification of a novel gene structure, as well as the essentiality of the 245 gene, 5283 gene, 2490 gene, 3963 gene or 4036 gene for normal plant growth and development, have been demonstrated for the first time in *Arabidopsis* using T-DNA insertion mutagenesis. Having established the essentiality of 245, 5283, 2490, 3963 or 4036 function in plants and having identified the genes encoding these essential activities, the inventors thereby provide an important and sought after tool for new herbicide development.

Arabidopsis insertional mutant lines segregating for seedling lethal mutations are identified as a first step in the identification of essential proteins. Starting with T2 seeds collected from single T1 plants containing T-DNA insertions in their genomes, those lines segregating homozygous seedling lethal seedlings are identified. These lines are found by placing seeds onto minimal plant growth media, which contains the fungicides benomyl and maxim, and screening for inviable seedlings after 7 and 14 days in the light at room

temperature. Inviable phenotypes include altered pigmentation or altered morphology. These phenotypes are observed either on plates directly or in soil following transplantation of seedlings.

When a line is identified as segregating a seedling lethal, it is determined if the resistance marker in the T-DNA co-segregates with the lethality (Errampalli et al. (1991) The Plant Cell, 3:149-157). Co-segregation analysis is done by placing the seeds on media containing the selective agent and scoring the seedlings for resistance or sensitivity to the agent. Examples of selective agents used are hygromycin or phosphinothricin. About 35 resistant seedlings are transplanted to soil and their progeny are examined for the segregation of the seedling lethal. In the case in which the T-DNA insertion disrupts an essential gene, there is co-segregation of the resistance phenotype and the seedling lethal phenotype in every plant. Therefore, in such a case, all resistant plants segregate seedling lethals in the next generation; this result indicates that each of the resistant plants is heterozygous for the DNA causing both phenotypes.

For those lines showing co-segregation of the T-DNA resistance marker and the seedling lethal phenotype, Southern analysis is performed as an initial step in the characterization of the molecular nature of each insertion. Southerns are done with genomic DNA isolated from heterozygotes and using probes capable of hybridizing with the T-DNA vector DNA. Using the results of the Southern analysis, appropriate restriction enzymes are chosen to perform plasmid rescue in order to molecularly clone *Arabidopsis* genomic DNA flanking one or both sides of the T-DNA insertion. Plasmids obtained in this manner are analyzed by restriction enzyme digestion to sort the plasmids into classes based on their digestion pattern. For each class of plasmid clone, the DNA sequence is determined. The resulting sequences are analyzed for the presence of non-T-DNA vector sequences. When such sequences are found, they are used to search DNA and protein databases using the BLAST and BLAST2 programs (Altschul et al. (1990) J Mol. Biol. 215: 403-410; Altschul et al (1997) Nucleic Acid Res. 25:3389-3402). Additional genomic and cDNA sequences for each gene are identified by standard molecular biology procedures.

The *Arabidopsis* 245 gene was identified by isolating DNA flanking the T-DNA border from the tagged seedling-lethal line #245. A region of the *Arabidopsis* DNA, flanking the T-DNA border, is 99% identical to the genomic survey sequence F17K7TR (accession # B24357). The inventors are the first to demonstrate that the 245 gene product is essential for normal growth and development in plants, as well as defining the function of the 245 gene product

through protein homology. The present invention discloses the cDNA nucleotide sequence of the *Arabidopsis* 245 gene as well as the amino acid sequence of the *Arabidopsis* 245 protein. The nucleotide sequence corresponding to the cDNA clone is set forth in SEQ ID NO:1, and the amino acid sequence encoding the protein is set forth in SEQ ID NO:2. The UTR sequence found 3' to SEQ ID NO:1 is set forth in SEQ ID NO:13. The nucleotide sequence corresponding to the partial genomic DNA is set forth in SEQ ID NO:12. The present invention also encompasses an isolated amino acid sequence derived from a plant, wherein said amino acid sequence is identical or substantially similar to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 1, wherein said amino acid sequence has 245 activity. Using BLAST and BLAST2 programs with the default settings, the sequence of the 245 gene shows similarity to peptide release factor 2 from numerous prokaryotic species. Notable species similarities include: *Escherichia coli* (RF-2) [Swiss-Prot accession #P07012]; *Salmonella typhimurium* (RF-2 Salty)[Swiss-Prot accession # P28353]; and *Mycobacterium tuberculosis* (RF-2: prfB)[Swiss-Prot accession #O05782]. Using GAP analysis of the following protein sequences with the 245 protein results in the following sequence identities with the 245 protein: *Escherichia coli* (RF-2) [Swiss-Prot accession #P07012](27.2% identity); *Salmonella typhimurium* (RF-2 Salty)[Swiss-Prot accession # P28353] (24.6% identity); and *Mycobacterium tuberculosis* (RF-2: prfB)[Swiss-Prot accession #O05782] (27.2% identity). In addition, *Synechocystis* (GenPept accession #BAA18577) (31.5% identity); and P1 clone MAB16, chromosome 5 of *Arabidopsis thaliana* (Accession #AB018112NID) (46.2% identity).

The *Arabidopsis* 5283 gene was identified by isolating DNA flanking the T-DNA border from the tagged seedling-lethal line #5283. A region of the *Arabidopsis* DNA, flanking the T-DNA border is identical to an internal region of a sequenced BAC of *Arabidopsis* (BAC T13D8, chromosome 1). This BAC clone contains 116,177 bp of sequence, of which a very small portion corresponds to the genomic region that contains the 5283 gene. Notwithstanding the BAC information, the inventors are the first to demonstrate that the 5283 gene product is essential for normal growth and development in plants, as well as defining the function of the 5283 gene product through protein homology. The present invention discloses the cDNA nucleotide sequence of the *Arabidopsis* 5283 gene as well as the amino acid sequence of the *Arabidopsis* 5283 protein. The nucleotide sequence corresponding to the cDNA clone is set forth in ***SEQ ID NO:3***, and the amino acid sequence encoding the protein

is set forth in ***SEQ ID NO:4***. The nucleotide sequence corresponding to the genomic DNA is set forth ***in SEQ ID NO: 14***. The nucleotide sequence corresponding to the 5' UTR from the cDNA sequence is set forth in ***SEQ ID NO:17***, and the nucleotide sequence corresponding to the 3'UTR from the cDNA sequence is set forth in ***SEQ ID NO:18***. The present invention also encompasses an isolated amino acid sequence derived from a plant, wherein said amino acid sequence is identical or substantially similar to the amino acid sequence encoded by the nucleotide sequence set forth in ***SEQ ID NO: 3***, wherein said amino acid sequence has 5283 activity. Using BLAST and BLAST2 programs with the default settings, the sequence of the 5283 protein shows similarity to SPBC119.13c from *S. pombe* [GENPEPT accession # CAA17928]; SAR DNA-binding proteins from plants [SARBP-1; Genbank accession # AF061962 and SARBP-2: Genbank accession # AF061963]; and prp31 and SIK1p (Nop56) from *S. cerevisiae* [PRP31: Swiss Prot accession # Q12460]. Using GAP analysis of the following protein sequences with the 5283 protein results in the following sequence identities with the 5283 protein: SPBC119.13c from *S. pombe* [GENPEPT accession # CAA17928] (40.5% identity); SAR DNA-binding proteins from plants [SARBP-1; Genbank accession # AF061962 (23.5% identity), and SARBP-2: Genbank accession # AF061963] (24.2% identity); and prp31 and SIK1p (Nop56) from *S. cerevisiae* [PRP31: Swiss Prot accession # Q12460] (24.1% identity). In addition, *Arabidopsis thaliana* (GENPEPT accession # AAC18800) results in 73.8% identity with the 5283 protein.

The *Arabidopsis* 2490 gene is identified by isolating DNA flanking the T-DNA border from the tagged seedling-lethal line #2490. *Arabidopsis* DNA flanking the T-DNA border is identical to an internal region of a sequenced P1 clone of *Arabidopsis* (P1 MTG13, chromosome 5). This P1 clone contains 50,641 bp of sequence, of which a small portion corresponds to the genomic region that contains the 2490 gene. The sequence of a 2490 cDNA containing the entire coding sequence for the 2490 protein is obtained by determining the sequence of the 144K24 EST clone (obtained from Michigan State University). Notwithstanding the BAC and EST sequence information, the inventors are the first to establish definitively the entire gene sequence, and to demonstrate that the 2490 gene product is essential for normal growth and development in plants, as well as defining the function of the 2490 gene product through protein homology. The present invention discloses the cDNA nucleotide sequence of the *Arabidopsis* 2490 gene as well as the

amino acid sequence of the *Arabidopsis* 2490 protein. The nucleotide sequence corresponding to the cDNA clone is set forth in ***SEQ ID NO:5***, and the amino acid sequence encoding the protein is set forth in ***SEQ ID NO:6***. The UTR sequence found 5' to ***SEQ ID NO:5*** is set forth in ***SEQ ID NO:20***, and the UTR sequence found 3' to ***SEQ ID NO:5*** is set forth in ***SEQ ID NO:21***. The nucleotide sequence corresponding to the genomic DNA is set forth in ***SEQ ID NO:19***. The present invention also encompasses an isolated amino acid sequence derived from a plant, wherein said amino acid sequence is identical or substantially similar to the amino acid sequence encoded by the nucleotide sequence set forth in ***SEQ ID NO: 5***, wherein said amino acid sequence has 2490 activity. Using BLAST and BLAST2 programs with the default settings, the sequence of the 2490 protein shows similarity to the Toc36 (bce42B) chloroplast envelope protein from *Brassica napus* (Ko et al. (1995) The Journal of Biological Chem. 270: 28601-28608; Wu et al. (1994) The Journal of Biological Chem. 269: 32264-32271; Pang et al. (1997) The Journal of Biological Chem. 272: 25623-25627). Using GAP analysis of the 2490 protein and the Toc36 (bce42B) chloroplast envelope protein from *Brassica napus* (Genbank accession #X79091) results in 81.7% identity with the 2490 protein.

The *Arabidopsis* 3963 gene is identified by isolating DNA flanking the T-DNA border from the tagged seedling-lethal line #3963. A region of the *Arabidopsis* DNA flanking the T-DNA border is 100% identical to the genomic sequence for P1 clone MDK4 on chromosome 5 (Genbank accession number AB010695). The inventors are the first to demonstrate that the 3963 gene product is essential for normal growth and development in plants, as well as defining the function of the 3963 gene product through protein homology. The present invention discloses the cDNA nucleotide sequence of the *Arabidopsis* 3963 gene as well as the amino acid sequence of the *Arabidopsis* 3963 protein. The nucleotide sequence corresponding to the cDNA clone is set forth in ***SEQ ID NO:7***, and the amino acid sequence encoding the protein is set forth in ***SEQ ID NO:8***. The UTR sequence found 3' to ***SEQ ID NO:7*** is set forth in ***SEQ ID NO:26***. The nucleotide sequence corresponding to the genomic DNA is set forth in ***SEQ ID NO:24***. The present invention also encompasses an isolated amino acid sequence derived from a plant, wherein said amino acid sequence is identical or substantially similar to the amino acid sequence encoded by the nucleotide sequence set forth in ***SEQ ID NO:7***, wherein said amino acid sequence has 3963 activity. Using BLAST and BLAST2 programs with the default settings, the sequence of the 3963 gene shows

similarity to a number of DNA repair proteins, including Rad32p from *Schizosaccharomyces pombe* (Genbank accession numberQ09683); hMre11 from *Homo sapiens* (Genbank accession number U37359); and Mre11p from *Saccharomyces cerevisiae* (Genbank accession number U60829). Using GAP analysis of the following protein sequences with the 3963 protein results in the following sequence identities with the 3963 protein: Rad32p from *Schizosaccharomyces pombe* (Genbank accession numberQ09683) (37.5% identity); hMre11 from *Homo sapiens* (Genbank accession number U37359) (39.4% identity); and Mre11p from *Saccharomyces cerevisiae* (Genbank accession number U60829) (34.7% identity).

The *Arabidopsis* 4036 gene is identified by isolating DNA flanking the T-DNA border from the tagged seedling-lethal line #4036. A region of the *Arabidopsis* DNA flanking the T-DNA border is 100% identical to the published genomic sequence for P1 clone MQB2, from chromosome 5 of *Arabidopsis* (Genbank accession # AB009053). The inventors are the first to demonstrate that the 4036 gene product is essential for normal growth and development in plants, as well as defining the function of the 4036 gene through protein homology. The present invention discloses the cDNA coding nucleotide sequence of the *Arabidopsis* 4036 gene as well as the amino acid sequence of the *Arabidopsis* 4036 protein. The nucleotide sequences corresponding to the cDNA of cv. Landsberg and that of two cultivars are set forth in ***SEQ ID NO:9*** and ***SEQ ID NO:28***, respectively. The corresponding amino acid sequences encoding the proteins are set forth in ***SEQ ID NO:10*** and ***SEQ ID NO:29***. The nucleotide sequence corresponding to the genomic DNA is set forth in ***SEQ ID NO:27***. Thirteen nucleotide differences are observed by comparing the cDNA clone, derived from cv. Landsberg, and the genomic sequence, derived from cv. Columbia, and these variations are listed below in Table 1.

Table 1. Nucleotide Differences Observed Between the 4036 cDNA Clone, from cv. Landsberg, and the 4036 Genomic Sequence, from cv. Columbia

Nucleotide #*	cv. Landsberg	cv. Columbia	Codon containing nucleotide difference (amino acid residue in cv. Landsberg and amino acid residue in cv. Columbia)**

115	G	A	GAT to AAT (Asp to Asn)
207	T	C	GTT to GTC (Val to Val)
273	C	T	TCC to TCT (Ser to Ser)
276	C	T	ATC to ATT (Ile to Ile)
321	T	C	TTT to TTC (Phe to Phe)
393	G	A	GCG to GCA (Ala to Ala)
485	T	A	CTA to CAA (Leu to Gln)
464	C	T	CCC to CTC (Pro to Leu)
559	A	C	AAG to CAG (Lys to Gln)
963	T	G	CCT to CCG (Pro to Pro)
1101	T	A	CCT to CCA (Pro to Pro)
1254	T	C	TTT to TTC (Phe to Phe)
1393	G	A	GAT to AAT (Asp to Asn)

**SEQ ID NO:9* used as a reference for nucleotide numbering

**Amino acid residues: Ala (alanine); Asn (asparagine); Asp (aspartic acid); Gln (glutamine); Ile (isoleucine); Leu (leucine); Lys (lysine); Phe (phenylalanine); Pro (proline); Ser (serine); and Val (valine)

The present invention also encompasses an isolated amino acid sequence derived from a plant, wherein said amino acid sequence is identical or substantially similar to the amino acid sequence encoded by the nucleotide sequence set forth in ***SEQ ID NO:9***, wherein said amino acid sequence has 4036 activity. Using BLAST and BLAST2 programs with the default settings, the sequence of the 4036 gene shows similarity to 1-deoxy-D-xylulose 5-phosphate reductoisomerase from a number of organisms including *Synechocystis* sp. (SWISS-PROTQ55663), *Bacillus subtilis* (SWISS-PROT O31753), and *Escherichia coli* (SWISS-PROT P45568) (Takahashi et al. (1998) Proc. Natl. Acad. Sci. USA, 95: 9879-9884). Using GAP analysis of the following protein sequences with the 4036 protein results in the following sequence identities with the 4036 protein: 1-deoxy-D-xylulose 5-phosphate reductoisomerase from *Synechocystis* sp. (SWISS-PROTQ55663) (66.1% identity); *Bacillus subtilis* (SWISS-PROT O31753) (45.4% identity); and *Escherichia coli* (SWISS-PROT P45568) (44.6% identity) (Takahashi et al. (1998) Proc. Natl. Acad. Sci. USA, 95: 9879-9884).

For recombinant production of 245, 5283, 2490, 3963 or 4036 activity in a host organism, a nucleotide sequence encoding a protein having 245, 5283, 2490, 3963 or 4036 activity is inserted into an expression cassette designed for the chosen host and introduced into the host where it is recombinantly produced. For example, SEQ ID NO:1 or SEQ ID NO:1 associated with ***SEQ ID NO:13*** as a 3' UTR, nucleotide sequences substantially similar to SEQ ID NO:1, or homologs of the 245 coding sequence can be used for the recombinant production of a protein having 245 activity. The choice of specific regulatory sequences such as promoter, signal sequence, 5' and 3' untranslated sequences, and enhancer appropriate for the chosen host is within the level of skill of the routineer in the art. The resultant molecule, containing the individual elements operably linked in proper reading frame, may be inserted into a vector capable of being transformed into the host cell. Suitable expression vectors and methods for recombinant production of proteins are well known for host organisms such as *E. coli*, yeast, and insect cells (see, *e.g.*, Luckow and Summers, *Bio/Technol. 6:* 47 (1988), and baculovirus expression vectors, e.g., those derived from the genome of *Autographica californica* nuclear polyhedrosis virus (AcMNPV). A preferred baculovirus/insect system is pAcHLT (Pharmingen, San Diego, CA) used to transfect *Spodoptera frugiperda* Sf9 cells (ATCC) in the presence of linear *Autographa californica* baculovirus DNA (Pharmigen, San Diego, CA). The resulting virus is used to infect HighFive *Tricoplusia ni* cells (Invitrogen, La Jolla, CA). In a similar fashion, recombinant production of 5283, 2490, 3963, or 4036 activity is obtained.

In a preferred embodiment, the nucleotide sequence encoding a protein having 245, 5283, 2490, 3963 or 4036 activity is derived from an eukaryote, such as a mammal, a fly or a yeast, but is preferably derived from a plant. In a further preferred embodiment, the nucleotide sequence is identical or substantially similar to the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:9 respectively or encodes a protein having 245, 5283, 2490, 3963 or 4036 activity, respectively, whose amino acid sequence is identical or substantially similar to the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10 respectively. The nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:9 encodes the *Arabidopsis* 245 protein, *Arabidopsis* 5283 protein, *Arabidopsis* 2490 protein, *Arabidopsis* 3963 protein or *Arabidopsis* 4036 protein, whose amino acid sequence is set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID

NO:6, SEQ ID NO:8 or SEQ ID NO:10 respectively. In another preferred embodiment, the nucleotide sequence is derived from a prokaryote, preferably a bacteria, e.g. *E. coli*.

Recombinantly produced protein having 245, 5283, 2490, 3963 or 4036 activity is isolated and purified using a variety of standard techniques. The actual techniques that may be used will vary depending upon the host organism used, whether the protein is designed for secretion, and other such factors familiar to the skilled artisan (*see, e.g.* chapter 16 of Ausubel, F. *et al.,* "Current Protocols in Molecular Biology", pub. by John Wiley & Sons, Inc. (1994).

Recombinantly produced proteins having 245, 5283, 2490, 3963 or 4036 activity are useful for a variety of purposes. For example, they can be used in *in vitro* assays to screen known herbicidal chemicals whose target has not been identified to determine if they inhibit 245, 5283, 2490, 3963 or 4036 activity. Such *in vitro* assays may also be used as more general screens to identify chemicals that inhibit such enzymatic activity and that are therefore novel herbicide candidates. Alternatively, recombinantly produced proteins having 245, 5283, 2490, 3963 or 4036 activity may be used to elucidate the complex structure of these molecules and to further characterize their association with known inhibitors in order to rationally design new inhibitory herbicides as well as herbicide tolerant forms of the enzymes.

In Vitro Inhibitor Assays: Discovery of Small Molecule Ligand that Interacts with the Gene Product of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9 respectively

Once a protein has been identified as a potential herbicide target, the next step is to develop an assay that allows screening a large number of chemicals to determine which ones interact with the protein. Although it is straightforward to develop assays for proteins of known function, developing assays with proteins of unknown functions is more difficult.

This difficulty can be overcome by using technologies that can detect interactions between a protein and a compound without knowing the biological function of the protein. A short description of three methods is presented, including fluorescence correlation spectroscopy, surface-enhanced laser desorption/ionization, and biacore technologies.

Fluorescence Correlation Spectroscopy (FCS) theory was developed in 1972 but it is only in recent years that the technology to perform FCS became available (Madge et al. (1972) Phys. Rev. Lett., 29: 705-708; Maiti et al. (1997) Proc. Natl. Acad. Sci. USA, 94: 11753-11757). FCS measures the average diffusion rate of a fluorescent molecule within a small sample volume. The sample size can be as low as 10^3 fluorescent molecules and the sample volume as low as the cytoplasm of a single bacterium. The diffusion rate is a function of the mass of the molecule and decreases as the mass increases. FCS can therefore be applied to protein-ligand interaction analysis by measuring the change in mass and therefore in diffusion rate of a molecule upon binding. . In a typical experiment, the target to be analyzed is expressed as a recombinant protein with a sequence tag, such as a poly-histidine sequence, inserted at the N or C-terminus. The expression takes place in *E. coli*, yeast or insect cells. The protein is purified by chromatography. For example, the poly-histidine tag can be used to bind the expressed protein to a metal chelate column such as Ni2+ chelated on iminodiacetic acid agarose. The protein is then labeled with a fluorescent tag such as carboxytetramethylrhodamine or BODIPY® (Molecular Probes, Eugene, OR). The protein is then exposed in solution to the potential ligand, and its diffusion rate is determined by FCS using instrumentation available from Carl Zeiss, Inc. (Thornwood, NY). Ligand binding is determined by changes in the diffusion rate of the protein.

Surface-Enhanced Laser Desorption/Ionization (SELDI) was invented by Hutchens and Yip during the late 1980's (Hutchens and Yip (1993) Rapid Commun. Mass Spectrom. 7: 576-580). When coupled to a time-of-flight mass spectrometer (TOF), SELDI provides a mean to rapidly analyze molecules retained on a chip. It can be applied to ligand-protein interaction analysis by covalently binding the target protein on the chip and analyze by MS the small molecules that bind to this protein (Worrall et al. (1998) Anal. Biochem. 70: 750-756). In a typical experiment, the target to be analyzed is expressed as described for FCS. The purified protein is then used in the assay without further preparation. It is bound to the SELDI chip either by utilizing the poly-histidine tag or by other interaction such as ion exchange or hydrophobic interaction. The chip thus prepared is then exposed to the potential ligand via, for example, a delivery system capable to pipet the ligands in a sequential manner (autosampler). The chip is then submitted to washes of increasing stringency, for example a series of washes with buffer solutions containing an increasing ionic strength. After each wash, the bound material is analyzed by submitting the chip to SELDI-TOF. Ligands that specifically bind the target will be identified by the stringency of the wash needed to elute them.

Biacore relies on changes in the refractive index at the surface layer upon binding of a ligand to a protein immobilized on the layer. In this system, a collection of small ligands is injected sequentially in a 2-5 ul cell with the immobilized protein. Binding is detected by surface plasmon resonance (SPR) by recording laser light refracting from the surface. In general, the refractive index change for a given change of mass concentration at the surface layer, is practically the same for all proteins and peptides, allowing a single method to be applicable for any protein (Liedberg et al. (1983) Sensors Actuators 4: 299-304; Malmquist (1993) Nature, 361: 186-187). In a typical experiment, the target to be analyzed is expressed as described for FCS. The purified protein is then used in the assay without further preparation. It is bound to the Biacore chip either by utilizing the poly-histidine tag or by other interaction such as ion exchange or hydrophobic interaction. The chip thus prepared is then exposed to the potential ligand via the delivery system incorporated in the instruments sold by Biacore (Uppsala, Sweden) to pipet the ligands in a sequential manner (autosampler). The SPR signal on the chip is recorded and changes in the refractive index indicate an interaction between the immobilized target and the ligand. Analysis of the signal kinetics on rate and off rate allows the discrimination between non-specific and specific interaction.

Also, an assay for small molecule ligands that interact with a polypeptide is an inhibitor assay. For example, such an inhibitor assay useful for identifying inhibitors of essential plant genes, such as 245, 5283, 2490, 3963, or 4036 genes, comprises the steps of:
a) reacting a plant 245, 5283, 2490, 3963, or 4036 protein and a substrate thereof in the presence of a suspected inhibitor of the protein's function;
b) comparing the rate of enzymatic activity in the presence of the suspected inhibitor to the rate of enzymatic activity under the same conditions in the absence of the suspected inhibitor; and
c) determining whether the suspected inhibitor inhibits the 245, 5283, 2490, 3963, or 4036 protein .

For example, the inhibitory effect on plant 245, 5283, 2490, 3963, or 4036 protein may be determined by a reduction or complete inhibition of 245, 5283, 2490, 3963, or 4036 activity in the assay. Such a determination may be made by comparing, in the presence and absence of the candidate inhibitor, the amount of substrate used or intermediate or product made during the reaction.

In one embodiment, a suspected herbicide, for example identified by *in vitro* screening, is applied to plants at various concentrations. The suspected herbicide is preferably sprayed on the plants. After application of the suspected herbicide, its effect on the plants, for example death or suppression of growth, is recorded.

In another embodiment, an *in vivo* screening assay for inhibitors of the 245, 5283, 2490, 3963 or 4036 activity uses transgenic plants, plant tissue, plant seeds or plant cells capable of overexpressing a nucleotide sequence having 245, 5283, 2490, 3963 or 4036 activity, wherein the 245, 5283, 2490, 3963 or 4036 gene product is enzymatically active in the transgenic plants, plant tissue, plant seeds or plant cells. The nucleotide sequence is preferably derived from an eukaryote, such as a yeast, but is preferably derived from a plant. In a further preferred embodiment, the nucleotide sequence is identical or substantially similar to the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9, or encodes an enzyme having 245, 5283, 2490, 3963 or 4036 activity, whose amino acid sequence is identical or substantially similar to the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10 respectively. In another preferred embodiment, the nucleotide sequence is derived from a prokaryote, preferably a bacteria, e.g. *E. coli.*

A chemical is then applied to the transgenic plants, plant tissue, plant seeds or plant cells and to the isogenic non-transgenic plants, plant tissue, plant seeds or plant cells, and the growth or viability of the transgenic and non-transformed plants, plant tissue, plant seeds or plant cells are determined after application of the chemical and compared. Compounds capable of inhibiting the growth of the non-transgenic plants, but not affecting the growth of the transgenic plants are selected as specific inhibitors of 245, 5283, 2490, 3963 or 4036 activity.

The present invention is further directed to plants, plant tissue, plant seeds, and plant cells tolerant to herbicides that inhibit the naturally occurring 245, 5283, 2490, 3963 or 4036 activity in these plants, wherein the tolerance is conferred by an altered 245, 5283, 2490, 3963 or 4036 activity respectively. Altered 245, 5283, 2490, 3963 or 4036 activity may be conferred upon a plant according to the invention by increasing expression of wild-type herbicide-sensitive 245, 5283, 2490, 3963 or 4036 gene, for example by providing additional wild-type 245, 5283, 2490, 3963 or 4036 genes and/or by overexpressing the

endogenous 245, 5283, 2490, 3963 or 4036 gene respectively, for example by driving expression with a strong promoter. Altered 245, 5283, 2490, 3963 or 4036 activity also may be accomplished by expressing nucleotide sequences that are substantially similar to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9 respectively or homologs thereof in a plant. Still further altered 245, 5283, 2490, 3963 or 4036 activity is conferred on a plant by expressing modified herbicide-tolerant 245, 5283, 2490, 3963 or 4036 genes respectively in the plant. Combinations of these techniques may also be used. Representative plants include any plants to which these herbicides are applied for their normally intended purpose. Preferred are agronomically important crops such as cotton, soybean, oilseed rape, sugar beet, maize, rice, wheat, barley, oats, rye, sorghum, millet, turf, forage, turf grasses, and the like.

Achieving altered 245 activity or 5283, 2490, 3963 4036 activity respectively through increased expression results in a level of 245 activity or 5283, 2490, 3963, 4036 activity respectively in the plant cell at least sufficient to overcome growth inhibition caused by the herbicide when applied in amounts sufficient to inhibit normal growth of control plants. The level of expressed enzyme generally is at least two times, preferably at least five times, and more preferably at least ten times the natively expressed amount. Increased expression may be due to multiple copies of a wild-type 245 gene or 5283, 2490, 3963 or 4036 gene respectively; multiple occurrences of the coding sequence within the gene (*i.e.* gene amplification) or a mutation in the non-coding, regulatory sequence of the endogenous gene in the plant cell. Plants having such altered gene activity can be obtained by direct selection in plants by methods known in the art (see, *e.g.* U.S. Patent No. 5,162,602, and U.S. Patent No. 4,761,373, and references cited therein). These plants also may be obtained by genetic engineering techniques known in the art. Increased expression of a herbicide-sensitive 245 gene or 5283, 2490, 3963 or 4036 gene respectively can also be accomplished by transforming a plant cell with a recombinant or chimeric DNA molecule comprising a promoter capable of driving expression of an associated structural gene in a plant cell operatively linked to a homologous or heterologous structural gene encoding the 245 protein or the 5283, 2490, 3963 or 4036 protein respectively or a homolog thereof. Preferably, the transformation is stable, thereby providing a heritable transgenic trait.

According to this embodiment, plants, plant tissue, plant seeds, or plant cells are stably transformed with a recombinant DNA molecule comprising a suitable promoter

functional in plants operatively linked to a coding sequence encoding a herbicide tolerant form of the 245, 5283, 2490, 3963 or 4036 protein respectively. A herbicide tolerant form of the enzyme has at least one amino acid substitution, addition or deletion that confers tolerance to a herbicide that inhibits the unmodified, naturally occurring form of the enzyme. The transgenic plants, plant tissue, plant seeds, or plant cells thus created are then selected by conventional selection techniques, whereby herbicide tolerant lines are isolated, characterized, and developed. Below are described methods for obtaining genes that encode herbicide tolerant forms of 245, 5283, 2490, 3963 or 4036 protein respectively:

One general strategy involves direct or indirect mutagenesis procedures on microbes. For instance, a genetically manipulatable microbe such as *E. coli* or *S. cerevisiae* may be subjected to random mutagenesis *in vivo* with mutagens such as UV light or ethyl or methyl methane sulfonate. Mutagenesis procedures are described, for example, in Miller, *Experiments in Molecular Genetics,* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1972); Davis *et al., Advanced Bacterial Genetics,* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1980); Sherman *et al., Methods in Yeast Genetics,* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1983); and U.S. Patent No. 4,975,374. The microbe selected for mutagenesis contains a normal, inhibitor-sensitive 245, 5283, 2490, 3963 or 4036 gene respectively and is dependent upon the activity conferred by this gene. The mutagenized cells are grown in the presence of the inhibitor at concentrations that inhibit the unmodified gene. Colonies of the mutagenized microbe that grow better than the unmutagenized microbe in the presence of the inhibitor (i.e. exhibit resistance to the inhibitor) are selected for further analysis. 245, 5283, 2490, 3963 or 4036 genes respectively conferring tolerance to the inhibitor are isolated from these colonies, either by cloning or by PCR amplification, and their sequences are elucidated. Sequences encoding altered gene products are then cloned back into the microbe to confirm their ability to confer inhibitor tolerance.

A method of obtaining mutant herbicide-tolerant alleles of a plant 245, 5283, 2490, 3963 or 4036 gene involves direct selection in plants. For example, the effect of a mutagenized 245, 5283, 2490, 3963 or 4036 gene on the growth inhibition of plants such as *Arabidopsis,* soybean, or maize is determined by plating seeds sterilized by art-recognized methods on plates on a simple minimal salts medium containing increasing concentrations of the inhibitor. Such concentrations are in the range of 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 110, 300, 1000 and 3000 parts per million (ppm). The lowest dose at which

significant growth inhibition can be reproducibly detected is used for subsequent experiments. Determination of the lowest dose is routine in the art.

Mutagenesis of plant material is utilized to increase the frequency at which resistant alleles occur in the selected population. Mutagenized seed material is derived from a variety of sources, including chemical or physical mutagenesis or seeds, or chemical or physical mutagenesis or pollen (Neuffer, In *Maize for Biological Research* Sheridan, ed. Univ. Press, Grand Forks, ND., pp. 61-64 (1982)), which is then used to fertilize plants and the resulting M_1 mutant seeds collected. Typically for *Arabidopsis*, M_2 seeds (Lehle Seeds, Tucson, AZ), which are progeny seeds of plants grown from seeds mutagenized with chemicals, such as ethyl methane sulfonate, or with physical agents, such as gamma rays or fast neutrons, are plated at densities of up to 10,000 seeds/plate (10 cm diameter) on minimal salts medium containing an appropriate concentration of inhibitor to select for tolerance. Seedlings that continue to grow and remain green 7-21 days after plating are transplanted to soil and grown to maturity and seed set. Progeny of these seeds are tested for tolerance to the herbicide. If the tolerance trait is dominant, plants whose seed segregate 3:1 / resistant:sensitive are presumed to have been heterozygous for the resistance at the M_2 generation. Plants that give rise to all resistant seed are presumed to have been homozygous for the resistance at the M_2 generation. Such mutagenesis on intact seeds and screening of their M2 progeny seed can also be carried out on other species, for instance soybean (*see, e.g.* U.S. Pat. No. 5,084,082). Alternatively, mutant seeds to be screened for herbicide tolerance are obtained as a result of fertilization with pollen mutagenized by chemical or physical means.

Confirmation that the genetic basis of the herbicide tolerance is a 245, 5283, 2490, 3963 or 4036 gene respectively is ascertained as exemplified below. First, alleles of the 245 5283, 2490, 3963 or 4036 gene respectively from plants exhibiting resistance to the inhibitor are isolated using PCR with primers based either upon the *Arabidopsis* cDNA coding sequences shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9 respectively or, more preferably, based upon the unaltered 245, 5283, 2490, 3963 or 4036 gene sequence from the plant used to generate tolerant alleles. After sequencing the alleles to determine the presence of mutations in the coding sequence, the alleles are tested for their ability to confer tolerance to the inhibitor on plants into which the putative tolerance-conferring alleles have been transformed. These plants can be either *Arabidopsis* plants or any other plant whose growth is susceptible to the 245, 5283, 2490, 3963 or 4036 inhibitors respectively . Second, the inserted 245, 5283, 2490, 3963 or 4036

genes are mapped relative to known restriction fragment length polymorphisms (RFLPs) (*See, for example,* Chang *et al. Proc. Natl. Acad, Sci, USA* 85: 6856-6860 (1988); Nam *et al., Plant Cell* 1: 699-705 (1989), cleaved amplified polymorphic sequences (CAPS) (Konieczny and Ausubel (1993) The Plant Journal, 4(2): 403-410), or SSLPs (Bell and Ecker (1994) Genomics, 19: 137-144). The 245, 5283, 2490, 3963 or 4036 inhibitor tolerance trait respectively is independently mapped using the same markers. When tolerance is due to a mutation in that 245, 5283, 2490, 3963 or 4036 gene respectively , the tolerance trait maps to a position indistinguishable from the position of the 245, 5283, 2490, 3963 or 4036 gene.

Another method of obtaining herbicide-tolerant alleles of a 245, 5283, 2490, 3963 or 4036 gene is by selection in plant cell cultures. Explants of plant tissue, *e.g.* embryos, leaf disks, etc. or actively growing callus or suspension cultures of a plant of interest are grown on medium in the presence of increasing concentrations of the inhibitory herbicide or an analogous inhibitor suitable for use in a laboratory environment. Varying degrees of growth are recorded in different cultures. In certain cultures, fast-growing variant colonies arise that continue to grow even in the presence of normally inhibitory concentrations of inhibitor. The frequency with which such faster-growing variants occur can be increased by treatment with a chemical or physical mutagen before exposing the tissues or cells to the inhibitor. Putative tolerance-conferring alleles of the 245, 5283, 2490, 3963 or 4036 gene respectively are isolated and tested as described in the foregoing paragraphs. Those alleles identified as conferring herbicide tolerance may then be engineered for optimal expression and transformed into the plant. Alternatively, plants can be regenerated from the tissue or cell cultures containing these alleles.

Still another method involves mutagenesis of wild-type, herbicide sensitive plant 245, 5283, 2490, 3963 or 4036 genes respectively in bacteria or yeast, followed by culturing the microbe on medium that contains inhibitory concentrations (i.e. sufficient to cause abnormal growth, inhibit growth or cause cell death) of the inhibitor, and then selecting those colonies that grow normally in the presence of the inhibitor. More specifically, a plant cDNA, such as the *Arabidopsis* cDNA encoding the 245, 5283, 2490, 3963 or 4036 protein respectively, is cloned into a microbe that otherwise lacks the 245 5283, 2490, 3963 or 4036 activity respectively. The transformed microbe is then subjected to *in vivo* mutagenesis or to *in vitro* mutagenesis by any of several chemical or enzymatic methods known in the art, e.g. sodium bisulfite (Shortle *et al., Methods Enzymol. 100:*457-468

(1983); methoxylamine (Kadonaga *et al., Nucleic Acids Res. 13:*1733-1745 (1985); oligonucleotide-directed saturation mutagenesis (Hutchinson *et al., Proc. Natl. Acad. Sci. USA, 83*:710-714 (1986); or various polymerase misincorporation strategies (see, e.g. Shortle et al., Proc. Natl. Acad. Sci. USA, 79:1588-1592 (1982); Shiraishi *et al., Gene 64*:313-319 (1988); and Leung *et al., Technique 1*:11-15 (1989). Colonies that grow normally in the presence of normally inhibitory concentrations of inhibitor are picked and purified by repeated restreaking. Their plasmids are purified and tested for the ability to confer tolerance to the inhibitor by retransforming them into the microbe lacking 245, 5283, 2490, 3963 or 4036 activity respectively. The DNA sequences of cDNA inserts from plasmids that pass this test are then determined.

Herbicide resistant 245, 5283, 2490, 3963 or 4036 proteins respectively are also obtained using methods involving *in vitro* recombination, also called DNA shuffling. By DNA shuffling, mutations, preferably random mutations, are introduced into nucleotide sequences encoding 245, 5283, 2490, 3963 or 4036 activity respectively. DNA shuffling also leads to the recombination and rearrangement of sequences within a 245, 5283, 2490, 3963 or 4036 gene respectively or to recombination and exchange of sequences between two or more different of 245, 5283, 2490, 3963 or 4036 genes respectively. These methods allow for the production of millions of mutated 245, 5283, 2490, 3963 or 4036 coding sequences respectively. The mutated genes, or shuffled genes, are screened for desirable properties, e.g. improved tolerance to herbicides and for mutations that provide broad spectrum tolerance to the different classes of inhibitor chemistry. Such screens are well within the skills of a routineer in the art.

In a preferred embodiment, a mutagenized 245, 5283, 2490, 3963 or 4036 gene respectively is formed from at least one template 245, 5283, 2490, 3963 or 4036 gene respectively, wherein the template 245 5283, 2490, 3963 or 4036 gene respectively has been cleaved into double-stranded random fragments of a desired size, and comprising the steps of adding to the resultant population of double-stranded random fragments one or more single or double-stranded oligonucleotides, wherein said oligonucleotides comprise an area of identity and an area of heterology to the double-stranded random fragments; denaturing the resultant mixture of double-stranded random fragments and oligonucleotides into single-stranded fragments; incubating the resultant population of single-stranded fragments with a polymerase under conditions which result in the annealing of said single-stranded fragments at said areas of identity to form pairs of annealed fragments, said areas

of identity being sufficient for one member of a pair to prime replication of the other, thereby forming a mutagenized double-stranded polynucleotide; and repeating the second and third steps for at least two further cycles, wherein the resultant mixture in the second step of a further cycle includes the mutagenized double-stranded polynucleotide from the third step of the previous cycle, and the further cycle forms a further mutagenized double-stranded polynucleotide, wherein the mutagenized polynucleotide is a mutated 245, 5283, 2490, 3963 or 4036 gene respectively having enhanced tolerance to a herbicide which inhibits naturally occurring 245, 5283, 2490, 3963 or 4036 activity respectively. In a preferred embodiment, the concentration of a single species of double-stranded random fragment in the population of double-stranded random fragments is less than 1% by weight of the total DNA. In a further preferred embodiment, the template double-stranded polynucleotide comprises at least about 100 species of polynucleotides. In another preferred embodiment, the size of the double-stranded random fragments is from about 5 bp to 5 kb. In a further preferred embodiment, the fourth step of the method comprises repeating the second and the third steps for at least 10 cycles. Such method is described e.g. in Stemmer et al. (1994) Nature 370: 389-391, in US Patent 5,605,793, US Patent 5,811,238 and in Crameri et al. (1998) Nature 391: 288-291, as well as in WO 97/20078, and these references are incorporated herein by reference.

In another preferred embodiment, any combination of two or more different 245 genes are mutagenized *in vitro* by a staggered extension process (StEP), as described e.g. in Zhao et al. (1998) Nature Biotechnology 16: 258-261. The two or more 245 genes are used as template for PCR amplification with the extension cycles of the PCR reaction preferably carried out at a lower temperature than the optimal polymerization temperature of the polymerase. In a similar fashion, the StEP is performed with the 5283,2490, 3963, or 4036 genes. For example, when a thermostable polymerase with an optimal temperature of approximately 72°C is used, the temperature for the extension reaction is desirably below 72°C, more desirably below 65°C, preferably below 60°C, more preferably the temperature for the extension reaction is 55°C. Additionally, the duration of the extension reaction of the PCR cycles is desirably shorter than usually carried out in the art, more desirably it is less than 30 seconds, preferably it is less than 15 seconds, more preferably the duration of the extension reaction is 5 seconds. Only a short DNA fragment is polymerized in each extension reaction, allowing template switch of the extension products between the starting DNA molecules after each cycle of denaturation and annealing, thereby generating diversity among the extension products. The optimal number of cycles in the PCR reaction depends

on the length of the 245, 5283, 2490, 3963 or 4036 genes respectively to be mutagenized but desirably over 40 cycles, more desirably over 60 cycles, preferably over 80 cycles are used. Optimal extension conditions and the optimal number of PCR cycles for every combination of 245, 5283, 2490, 3963 or 4036 genes respectively are determined as described in using procedures well-known in the art. The other parameters for the PCR reaction are essentially the same as commonly used in the art. The primers for the amplification reaction are preferably designed to anneal to DNA sequences located outside of the 245, 5283, 2490, 3963 or 4036 genes, e.g. to DNA sequences of a vector comprising the 245, 5283, 2490, 3963 or 4036 genes respectively, whereby the different 245, 5283, 2490, 3963 or 4036 genes respectively used in the PCR reaction are preferably comprised in separate vectors. The primers desirably anneal to sequences located less than 500 bp away from 245, 5283, 2490, 3963 or 4036 respectively sequences, preferably less than 200 bp, more preferably less than 120 bp away from the 245, 5283, 2490, 3963 or 4036 sequences respectively. Preferably, the 245, 5283, 2490, 3963 or 4036 sequences respectively are surrounded by restriction sites, which are included in the DNA sequence amplified during the PCR reaction, thereby facilitating the cloning of the amplified products into a suitable vector. In another preferred embodiment, fragments of 245 5283, 2490, 3963 or 4036 genes respectively having cohesive ends are produced as described in WO 98/05765. The cohesive ends are produced by ligating a first oligonucleotide corresponding to a part of a 245, 5283, 2490, 3963 or 4036 gene respectively to a second oligonucleotide not present in the gene or corresponding to a part of the gene not adjoining to the part of the gene corresponding to the first oligonucleotide, wherein the second oligonucleotide contains at least one ribonucleotide. A double-stranded DNA is produced using the first oligonucleotide as template and the second oligonucleotide as primer. The ribonucleotide is cleaved and removed. The nucleotide(s) located 5' to the ribonucleotide is also removed, resulting in double-stranded fragments having cohesive ends. Such fragments are randomly reassembled by ligation to obtain novel combinations of gene sequences.

Any 245, 5283, 2490, 3963 or 4036 gene respectively or any combination of 245 5283, 2490, 3963 or 4036 genes is used for *in vitro* recombination in the context of the present invention, for example, a 245, 5283, 2490, 3963 or 4036 gene respectively derived from a plant, such as, e.g. *Arabidopsis thaliana*, e.g. a 245, 5283, 2490, 3963 or 4036 gene respectively set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9 respectively, or a 245-like, 5283-like, 2490-like, 3963-like or 4036-like gene respectively from *E. coli* (Craigen et al. (1985) Proc Natl Acad Sci, 82: 3616-3620; Craigen

and Caskey (1987) Biochimie, 69: 1031-1041; Ito et al. (1998) Proc Natl Acad Sci, 95: 8165-8169), all of which are incorporated herein by reference. Whole 245, 5283, 2490, 3963 or 4036 genes respectively or portions thereof are used in the context of the present invention. The library of mutated 245, 5283, 2490, 3963 or 4036 genes respectively obtained by the methods described above are cloned into appropriate expression vectors and the resulting vectors are transformed into an appropriate host, for example an algae like *Chlamydomonas*, a yeast or a bacteria. An appropriate host is preferably a host that otherwise lacks 245, 5283, 2490, 3963 or 4036 activity, for example *E. coli*. Host cells transformed with the vectors comprising the library of mutated 245, 5283, 2490, 3963 or 4036 genes respectively are cultured on medium that contains inhibitory concentrations of the inhibitor and those colonies that grow in the presence of the inhibitor are selected. Colonies that grow in the presence of normally inhibitory concentrations of inhibitor are picked and purified by repeated restreaking. Their plasmids are purified and the DNA sequences of cDNA inserts from plasmids that pass this test are then determined.

An assay for identifying a modified 245, 5283, 2490, 3963 or 4036 gene respectively that is tolerant to an inhibitor may be performed in the same manner as the assay to identify inhibitors of the 245, 5283, 2490, 3963 or 4036 activity respectively (Inhibitor Assay, above) with the following modifications: First, a mutant 245, 5283, 2490, 3963 or 4036 protein respectively is substituted in one of the reaction mixtures for the wild-type 245 5283, 2490, 3963 or 4036 protein respectively of the inhibitor assay. Second, an inhibitor of wild-type enzyme is present in both reaction mixtures. Third, mutated activity (activity in the presence of inhibitor and mutated enzyme) and unmutated activity (activity in the presence of inhibitor and wild-type enzyme) are compared to determine whether a significant increase in enzymatic activity is observed in the mutated activity when compared to the unmutated activity. Mutated activity is any measure of activity of the mutated enzyme while in the presence of a suitable substrate and the inhibitor. Unmutated activity is any measure of activity of the wild-type enzyme while in the presence of a suitable substrate and the inhibitor.

In addition to being used to create herbicide-tolerant plants, genes encoding herbicide tolerant 245, 5283, 2490, 3963 or 4036 protein respectively can also be used as selectable markers in plant cell transformation methods. For example, plants, plant tissue, plant seeds, or plant cells transformed with a heterologous DNA sequence can also be transformed with a sequence encoding an altered 245, 5283, 2490, 3963 or 4036 activity respectively capable of being expressed by the plant. The transformed cells are transferred

to medium containing an inhibitor of the enzyme in an amount sufficient to inhibit the growth or survivability of plant cells not expressing the modified coding sequence, wherein only the transformed cells will grow. The method is applicable to any plant cell capable of being transformed with a modified 245, 5283, 2490, 3963 or 4036 gene, and can be used with any heterologous DNA sequence of interest. Expression of the heterologous DNA sequence and the modified gene can be driven by the same promoter functional in plant cells, or by separate promoters.

X.

A wild type or herbicide-tolerant form of the 245, 5283, 2490, 3963 or 4036 gene respectively, or homologs thereof, can be incorporated in plant or bacterial cells using conventional recombinant DNA technology. Generally, this involves inserting a DNA molecule encoding the 245, 5283, 2490, 3963 or 4036 gene respectively into an expression system to which the DNA molecule is heterologous (i.e., not normally present) using standard cloning procedures known in the art. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences in a host cell containing the vector. A large number of vector systems known in the art can be used, such as plasmids, bacteriophage viruses and other modified viruses. The components of the expression system may also be modified to increase expression. For example, truncated sequences, nucleotide substitutions, nucleotide optimization or other modifications may be employed. Expression systems known in the art can be used to transform virtually any crop plant cell under suitable conditions. A heterologous DNA sequence comprising a wild-type or herbicide-tolerant form of the 245, 5283, 2490, 3963 or 4036 gene respectively is preferably stably transformed and integrated into the genome of the host cells. In another preferred embodiment, the heterologous DNA sequence comprising a wild-type or herbicide-tolerant form of the 245, 5283, 2490, 3963 or 4036 gene respectively located on a self-replicating vector. Examples of self-replicating vectors are viruses, in particular gemini viruses. Transformed cells can be regenerated into whole plants such that the chosen form of the 245, 5283, 2490, 3963 or 4036 gene respectively confers herbicide tolerance in the transgenic plants.

Gene sequences intended for expression in transgenic plants are first assembled in expression cassettes behind a suitable promoter expressible in plants. The expression

cassettes may also comprise any further sequences required or selected for the expression of the heterologous DNA sequence. Such sequences include, but are not restricted to, transcription terminators, extraneous sequences to enhance expression such as introns, vital sequences, and sequences intended for the targeting of the gene product to specific organelles and cell compartments. These expression cassettes can then be easily transferred to the plant transformation vectors described *infra*. The following is a description of various components of typical expression cassettes.

The selection of the promoter used in expression cassettes will determine the spatial and temporal expression pattern of the heterologous DNA sequence in the plant transformed with this DNA sequence. Selected promoters will express heterologous DNA sequences in specific cell types (such as leaf epidermal cells, mesophyll cells, root cortex cells) or in specific tissues or organs (roots, leaves or flowers, for example) and the selection will reflect the desired location of accumulation of the gene product. Alternatively, the selected promoter may drive expression of the gene under various inducing conditions. Promoters vary in their strength, i.e., ability to promote transcription. Depending upon the host cell system utilized, any one of a number of suitable promoters known in the art can be used. For example, for constitutive expression, the CaMV 35S promoter, the rice actin promoter, or the ubiquitin promoter may be used. For regulatable expression, the chemically inducible PR-1 promoter from tobacco or *Arabidopsis* may be used (*see, e.g.*, U.S. Patent No. 5,689,044).

A variety of transcriptional terminators are available for use in expression cassettes. These are responsible for the termination of transcription beyond the heterologous DNA sequence and its correct polyadenylation. Appropriate transcriptional terminators are those that are known to function in plants and include the CaMV 35S terminator, the *tml* terminator, the nopaline synthase terminator and the pea *rbcS* E9 terminator. These can be used in both monocotyledonous and dicotyledonous plants.

Numerous sequences have been found to enhance gene expression from within the transcriptional unit and these sequences can be used in conjunction with the genes of this invention to increase their expression in transgenic plants. For example, various intron sequences such as introns of the maize *Adhl* gene have been shown to enhance expression, particularly in monocotyledonous cells. In addition, a number of non-translated

leader sequences derived from viruses are also known to enhance expression, and these are particularly effective in dicotyledonous cells.

The coding sequence of the selected gene optionally is genetically engineered by altering the coding sequence for optimal expression in the crop species of interest. Methods for modifying coding sequences to achieve optimal expression in a particular crop species are well known (see, *e.g.* Perlak *et al., Proc. Natl. Acad. Sci. USA 88:* 3324 (1991); and Koziel *et al., Bio/technol. 11:* 194 (1993); Fennoy and Bailey-Serres. *Nucl. Acids Res.* 21: 5294-5300 (1993). Methods for modifying coding sequences by taking into account codon usage in plant genes and in higher plants, green algae, and cyanobacteria are well known (see table 4 in: Murray et al. *Nucl. Acids Res.* 17: 477-498 (1989); Campbell and Gowri *Plant Physiol.* 92: 1-11(1990).

Various mechanisms for targeting gene products are known to exist in plants and the sequences controlling the functioning of these mechanisms have been characterized in some detail. For example, the targeting of gene products to the chloroplast is controlled by a signal sequence found at the amino terminal end of various proteins which is cleaved during chloroplast import to yield the mature protein (*e.g.* Comai *et al.* J. Biol. Chem. 263: 15104-15109 (1988)). Other gene products are localized to other organelles such as the mitochondrion and the peroxisome (*e.g.* Unger *et al.* Plant Molec. Biol. 13: 411-418 (1989)). The cDNAs encoding these products can also be manipulated to effect the targeting of heterologous products encoded by DNA sequences to these organelles. In addition, sequences have been characterized which cause the targeting of products encoded by DNA sequences to other cell compartments. Amino terminal sequences are responsible for targeting to the ER, the apoplast, and extracellular secretion from aleurone cells (Koehler & Ho, Plant Cell 2: 769-783 (1990)). Additionally, amino terminal sequences in conjunction with carboxy terminal sequences are responsible for vacuolar targeting of gene products (Shinshi *et al.* Plant Molec. Biol. 14: 357-368 (1990)). By the fusion of the appropriate targeting sequences described above to heterologous DNA sequences of interest it is possible to direct this product to any organelle or cell compartment.

Numerous transformation vectors available for plant transformation are known to those of ordinary skill in the plant transformation arts, and the genes pertinent to this invention can be used in conjunction with any such vectors. The selection of vector will depend upon the preferred transformation technique and the target species for transformation. For certain target species, different antibiotic or herbicide selection markers may be preferred. Selection markers used routinely in transformation include the *nptII* gene, which confers resistance to kanamycin and related antibiotics (Messing & Vierra. Gene 19: 259-268 (1982); Bevan et al., Nature 304:184-187 (1983)), the *bar* gene, which confers resistance to the herbicide phosphinothricin (White et al., Nucl. Acids Res 18: 1062 (1990), Spencer et al. Theor. Appl. Genet 79: 625-631 (1990)), the *hph* gene, which confers resistance to the antibiotic hygromycin (Blochinger & Diggelmann, Mol Cell Biol 4: 2929-2931), and the *dhfr* gene, which confers resistance to methotrexate (Bourouis et al., EMBO J. 2(7): 1099-1104 (1983)), and the EPSPS gene, which confers resistance to glyphosate (U.S. Patent Nos. 4,940,935 and 5,188,642).

Many vectors are available for transformation using *Agrobacterium tumefaciens*. These typically carry at least one T-DNA border sequence and include vectors such as pBIN19 (Bevan, Nucl. Acids Res. (1984)). Typical vectors suitable for *Agrobacterium* transformation include the binary vectors pCIB200 and pCIB2001, as well as the binary vector pCIB10 and hygromycin selection derivatives thereof. (*See*, for example, U.S. Patent No. 5,639,949).

Transformation without the use of *Agrobacterium tumefaciens* circumvents the requirement for T-DNA sequences in the chosen transformation vector and consequently vectors lacking these sequences can be utilized in addition to vectors such as the ones described above which contain T-DNA sequences. Transformation techniques that do not rely on *Agrobacterium* include transformation via particle bombardment, protoplast uptake (*e.g.* PEG and electroporation) and microinjection. The choice of vector depends largely on the preferred selection for the species being transformed. Typical vectors suitable for non-

Agrobacterium transformation include pCIB3064, pSOG19, and pSOG35. (*See*, for example, U.S. Patent No. 5,639,949).

Once the coding sequence of interest has been cloned into an expression system, it is transformed into a plant cell. Methods for transformation and regeneration of plants are well known in the art. For example, Ti plasmid vectors have been utilized for the delivery of foreign DNA, as well as direct DNA uptake, liposomes, electroporation, micro-injection, and microprojectiles. In addition, bacteria from the genus *Agrobacterium* can be utilized to transform plant cells.

Transformation techniques for dicotyledons are well known in the art and include *Agrobacterium*-based techniques and techniques that do not require *Agrobacterium*. Non-*Agrobacterium* techniques involve the uptake of exogenous genetic material directly by protoplasts or cells. This can be accomplished by PEG or electroporation mediated uptake, particle bombardment-mediated delivery, or microinjection. In each case the transformed cells are regenerated to whole plants using standard techniques known in the art.

Transformation of most monocotyledon species has now also become routine. Preferred techniques include direct gene transfer into protoplasts using PEG or electroporation techniques, particle bombardment into callus tissue, as well as *Agrobacterium*-mediated transformation.

In another preferred embodiment, a nucleotide sequence encoding a polypeptide having 245, 5283, 2490, 3963, or 4036 activity is directly transformed into the plastid genome. Plastid expression, in which genes are inserted by homologous recombination into the several thousand copies of the circular plastid genome present in each plant cell, takes advantage of the enormous copy number advantage over nuclear-expressed genes to permit expression levels that can readily exceed 10% of the total soluble plant protein. In a preferred embodiment, the nucleotide sequence is inserted into a plastid targeting vector and transformed into the plastid genome of a desired plant host. Plants homoplasmic for plastid genomes containing the nucleotide sequence are obtained, and are preferentially capable of high expression of the nucleotide sequence.

Plastid transformation technology is for example extensively described in U.S. Patent Nos. 5,451,513, 5,545,817, 5,545,818, and 5,877,462 in PCT application no. WO 95/16783 and WO 97/32977, and in McBride *et al.* (1994) Proc. Natl. Acad. Sci. USA 91, 7301-7305, all

incorporated herein by reference in their entirety. The basic technique for plastid transformation involves introducing regions of cloned plastid DNA flanking a selectable marker together with the nucleotide sequence into a suitable target tissue, e.g., using biolistics or protoplast transformation (e.g., calcium chloride or PEG mediated transformation). The 1 to 1.5 kb flanking regions, termed targeting sequences, facilitate homologous recombination with the plastid genome and thus allow the replacement or modification of specific regions of the plastome. Initially, point mutations in the chloroplast 16S rRNA and rps12 genes conferring resistance to spectinomycin and/or streptomycin are utilized as selectable markers for transformation (Svab, Z., Hajdukiewicz, P., and Maliga, P. (1990) Proc. Natl. Acad. Sci. USA 87, 8526-8530; Staub, J. M., and Maliga, P. (1992) Plant Cell 4, 39-45). The presence of cloning sites between these markers allowed creation of a plastid targeting vector for introduction of foreign genes (Staub, J.M., and Maliga, P. (1993) *EMBO J.* 12, 601-606). Substantial increases in transformation frequency are obtained by replacement of the recessive rRNA or r-protein antibiotic resistance genes with a dominant selectable marker, the bacterial *aadA* gene encoding the spectinomycin-detoxifying enzyme aminoglycoside-3'-adenyltransferase (Svab, Z., and Maliga, P. (1993) *Proc. Natl. Acad. Sci. USA* 90, 913-917). Other selectable markers useful for plastid transformation are known in the art and encompassed within the scope of the invention.

The wild-type or altered form of a 245, 5283, 2490, 3963 or 4036 gene respectively of the present invention can be utilized to confer herbicide tolerance to a wide variety of plant cells, including those of gymnosperms, monocots, and dicots. Although the gene can be inserted into any plant cell falling within these broad classes, it is particularly useful in crop plant cells, such as rice, wheat, barley, rye, corn, potato, carrot, sweet potato, sugar beet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum and sugarcane.

The high-level expression of a wild-type 245, 5283, 2490, 3963 or 4036 gene respectively and/or the expression of herbicide-tolerant forms of a 245, 5283, 2490, 3963 or 4036 gene respectively conferring herbicide tolerance in plants, in combination with other characteristics important for production and quality, can be incorporated into plant lines through breeding approaches and techniques known in the art.

Where a herbicide tolerant 245, 5283, 2490, 3963 or 4036 gene allele respectively is obtained by direct selection in a crop plant or plant cell culture from which a crop plant can be regenerated, it is moved into commercial varieties using traditional breeding techniques to develop a herbicide tolerant crop without the need for genetically engineering the allele and transforming it into the plant.

The invention will be further described by reference to the following detailed examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified.

EXAMPLES

Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by Sambrook, *et al.*, Molecular Cloning, eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989) and by T.J. Silhavy, M.L. Berman, and L.W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984) and by Ausubel, F.M. *et al.*, Current Protocols in Molecular Biology, pub. by Greene Publishing Assoc. and Wiley-Interscience (1987), Reiter, et al., Methods in *Arabidopsis* Research, World Scientific Press (1992), and Schultz et al., Plant Molecular Biology Manual, Kluwer Academic Publishers (1998). These references describe the standard techniques used for all steps in tagging and cloning genes from T-DNA mutagenized populations of *Arabidopsis*: plant infection and transformation; screening for the identification of seedling mutants; cosegregation analysis; and plasmid rescue.

Example 1: Sequence Analysis of Tagged Seedling – Lethal Line #245 From the T-DNA Mutagenized Population of *Arabidopsis*

The plasmid rescue technique is used to molecularly clone *Arabidopsis* genomic DNA flanking one or both sides of T-DNA insertions resulting from T-DNA mutagenesis. Plasmids obtained in this manner are analyzed by restriction enzyme digestion to sort the plasmids into classes based on their digestion pattern. For each class of plasmid clone, the DNA sequence is determined. The resulting sequences are analyzed for the presence of non-T-DNA vector sequences. The plasmids recovered from the plasmid rescue protocol are sequenced using the slp346for primer (SEQ ID NO:11). Primer slp346for provides information on the flanking sequence immediately adjacent to the left T-DNA border. Plasmid rescue is validated by PCR of genomic DNA from a homozygote for the 245 mutation. This PCR experiment uses a primer anchored in the predicted flanking sequence and the slp346for primer (anchored in the T-DNA insertion). Finding a PCR product of the size expected based on the sequence of the plasmid rescued clone confirms a valid rescue. The sequence obtained from primer slp346for is used in a BLASTx search against nucleotide sequence databases (Altschul *et al.* (1990) J Mol. Biol. 215:403-410; Altschul *et al.* (1997) Nucleic Acids Res. 25: 3389-3402.). The BLAST search results show that the recovered plant flanking sequence shows a high level of similarity to numerous prokaryotic

peptide release factor two proteins. The BLAST results indicate that the T-DNA insertion has occurred in the ORF of the first identified plant derived peptide release factor two. A DNA fragment that includes peptide release factor sequence similarity is isolated by amplification of *Arabidopsis* genomic DNA using the polymerase chain reaction. This fragment is used to probe an *Arabidopsis* cDNA library in the λYES vector (Elledge *et al.* (1991) Proc. Natl. Acad. Sci. 88:1731-1735). Positive phage clones are isolated and characterized using standard molecular biology techniques. The resultant cDNA clones are excised from the phage and the nucleotide sequence is determined. The DNA sequence is shown in SEQ ID NO:1. The deduced amino acid sequence is analyzed using the BLASTx search against nucleotide sequence databases (Altschul *et al.* (1990) J Mol. Biol. 215:403-410; Altschul *et al.* (1997) Nucleic Acids Res. 25: 3389-3402). The BLAST search results show that the recovered 245 cDNA shows sequence similarity to the same set of prokaryotic peptide release factors.

Example 2: Sequence Analysis of Tagged Seedling – Lethal Line #5283 From the T-DNA Mutagenized Population of *Arabidopsis*

The plasmid rescue technique is used to molecularly clone *Arabidopsis* genomic DNA flanking one or both sides of T-DNA insertions resulting from T-DNA mutagenesis. Plasmids obtained in this manner are analyzed by restriction enzyme digestion to sort the plasmids into classes based on their digestion pattern. For each class of plasmid clone, the DNA sequence is determined. The resulting sequences are analyzed for the presence of non-T-DNA vector sequences. The plasmids recovered from the plasmid rescue protocol are sequenced using the slp346for primer (***SEQ ID NO:11***). Primer slp346for provides information on the flanking sequence immediately adjacent to the left T-DNA border. Plasmid rescue is validated by PCR of genomic DNA from a heterozygote for the 5283 mutation. This PCR experiment uses a primer anchored in the predicted flanking sequence and the slp328 primer ***(SEQ ID NO:15***) (anchored in the T-DNA insertion). Finding a PCR product of the size expected based on the sequence of the plasmid rescued clone confirms a valid rescue.

The sequence obtained from primer SLP346for is used in a BLASTn search against nucleotide sequence databases (Altschul *et al.* (1990) J Mol. Biol. 215:403-410; Altschul *et al.* (1997) Nucleic Acids Res. 25: 3389-3402.). The BLAST search results show that the

recovered sequence is identical to genomic DNA located in *Arabidopsis* chromosome I, BAC T13D8 (Genbank accession number AC004473). Primer LW60 (***SEQ ID NO:16***), the reverse complement to nucleotides #32,964-32,987 in the BAC T13D8 sequence (5'-aaacgcttaccatatctctttcta-3'), is designed and used to determine the sequence downstream of the T-DNA insert; this experiment identifies the junction of the right border. The region of genomic DNA where the T-DNA insertion occurred includes bases #32,879 through #32,885 of the annotated BAC T13D8 sequence, resulting in a six-base deletion. This insertion occurs 90 nucleotides upstream of the sequence annotated on BAC T13D8 as encoding a protein similar to S. *cerevisiae* SIK1P protein (Genbank accession number U20237). A DNA fragment that includes bases #33,025 through bases #34,338 of the BAC T13D8 sequence is isolated by amplification of *Arabidopsis* genomic DNA using the polymerase chain reaction. This fragment is used to probe an *Arabidopsis* cDNA library in the IYES vector (Elledge *et al.* (1991) Proc. Natl. Acad. Sci. 88:1731-1735). Positive phage clones are isolated and characterized using standard molecular biology techniques. The resultant cDNA clones are excised from the phage and the nucleotide sequence is determined. One full-length clone is identified. The deduced amino acid sequence is analyzed using the tBLASTn search against nucleotide sequence databases (Altschul *et al.* (1990) J Mol. Biol. 215:403-410; Altschul *et al.* (1997) Nucleic Acids Res. 25: 3389-3402). The BLAST search results show that the recovered 5283 cDNA sequence is derived from the same genomic sequence located in *Arabidopsis* chromosome I, BAC T13D8. The intron/exon boundaries of the cDNA sequence are the same as those predicted for the *Arabidopsis* SIK1P homolog (Genbank accession number AC004473), with the following exceptions. The initiator codon for the 5283 cDNA is encoded by bases #32975 through #32977, followed immediately by an intron at bases #32978 through #33199.

Example 3: Sequence Analysis of Tagged Seedling – Lethal Line #2490 From the T-DNA Mutagenized Population of *Arabidopsis*

The plasmid rescue technique is used to molecularly clone *Arabidopsis* genomic DNA flanking one or both sides of T-DNA insertions resulting from T-DNA mutagenesis. Plasmids obtained in this manner are analyzed by restriction enzyme digestion to sort the plasmids into classes based on their digestion pattern. For each class of plasmid clone, the DNA sequence is determined. The resulting sequences are analyzed for the presence of

non-T-DNA vector sequences. The plasmids recovered from the plasmid rescue protocol are sequenced using the SLP346for primer (5' GCGGACATCTACATTTTTGA 3': ***SEQ ID NO:11***). Primer SLP346for provides information on the flanking sequence immediately adjacent to the left T-DNA border. Clones for both ends of the T-DNA insertion are recovered as plasmids containing left T-DNA border. Plasmid rescue is validated by Southern blot analysis comparing genomic DNA from a plant heterozygous for the 2490 mutation with genomic DNA from a plant homozygous for the wild-type 2490 gene. The probe for the Southern blot is prepared from a PCR product generated with the SLP369 (5' CAGACCACAATACCTTCAAAAATA 3': ***SEQ ID NO:22***) and SLP370 (5' CCATTGTGTCTCCCTCCCGCTGTT 3': ***SEQ ID NO:23***) primers. Finding an additional *Bam*H1 fragment in the 2490 heterozygote confirms a valid rescue.

The sequences obtained from the above clones are used in a BLASTn search against nucleotide sequence databases (Altschul et al. (1990) J Mol. Biol. 215: 403-410; Altschul et al (1997) Nucleic Acids Res. 25: 3389-3402). The search results show that the recovered sequences are identical to genomic DNA from *Arabidopsis* chromosome 5 P1 clone MTG13 (Genbank # AB008270). When the region of genomic DNA where the insertion event occurred is used in a BLASTn search of the Genbank EST database, four sequences derived from the ends of two ESTs, 144K24 (144K24 T7 Genbank #T76608 and 144K24XP Genbank #AA404903) and GBGF153 (5' end Genbank #F15182 and 3' end Genbank #F15181) are identified. The complete sequence of the 144K24 EST is determined and this sequence encodes the full open reading frame (ORF) for the 2490 gene. BLAST analysis of this EST indicates that the 2490 protein has sequence similarity with the *Brassica napus* Toc36 protein (Genbank #X79091; Ko et al. (1995) The Journal of Biological Chem. 270: 28601-28608; Wu et al. (1994) The Journal of Biological Chem. 269: 32264-32271; Pang et al. (1997) The Journal of Biological Chem. 272: 25623-25627). The Toc36 protein has also been referred to as bce44B, Com44, and Cim44. Because the genomic DNA that contains the 2490 ORF was not annotated correctly until now, the inventors are the first to provide experimental documentation of the correct ORF and sequence similarity for the 2490 gene.

Example 4: Sequence Analysis of Tagged Seedling – Lethal Line #3963 From the T-DNA Mutagenized Population of *Arabidopsis*

The plasmid rescue technique is used to molecularly clone *Arabidopsis* genomic DNA flanking one or both sides of T-DNA insertions resulting from T-DNA mutagenesis. Plasmids obtained in this manner are analyzed by restriction enzyme digestion to sort the plasmids into classes based on their digestion pattern. For each class of plasmid clone, the DNA sequence is determined. The resulting sequences are analyzed for the presence of non-T-DNA vector sequences. The plasmids recovered from the plasmid rescue protocol are sequenced using the -21 primer (5' TGTAAAACGACGGCCAGT 3'; ***SEQ ID NO:25***). Primer -21 provides information on the flanking sequence immediately adjacent to the right T-DNA border. Plasmid rescue is validated by PCR of genomic DNA from a heterozygote for the 3963 mutation. This PCR experiment uses a primer anchored in the predicted flanking sequence and the -21 primer (anchored in the T-DNA insertion). Finding a PCR product of the size expected based on the sequence of the plasmid rescued clone confirms a valid rescue. The sequence obtained from primer -21 is used in a BLASTn search against nucleotide sequence databases (Altschul *et al.* (1990) J Mol. Biol. 215:403-410; Altschul *et al.* (1997) Nucleic Acids Res. 25: 3389-3402.). The BLAST search results show that the recovered plant flanking sequence is 100% identical to the genomic sequence for P1 clone MDK4 on chromosome 5 (Genbank accession number AB010695). The T-DNA insertion occurred at base # 36342 of the annotated P1 clone MDK4 sequence, in the gene identified as MDK4.6. A tBLASTX analysis of the recovered flanking sequence shows sequence similarity to Mre11p, a DNA repair protein from *Sacchromyces cerevisiae* (Genbank accession number U60829). A fragment that encodes part of the *Arabidopsis* 3963 protein is isolated by amplification of *Arabidopsis* genomic DNA using the polymerase chain reaction. This fragment is used to probe an *Arabidopsis* cDNA library in the λYES vector (Elledge *et al.* (1991) Proc. Natl. Acad. Sci. 88:1731-1735). Positive phage clones are isolated and characterized using standard molecular biology techniques. The resultant cDNA clones are excised from the phage and the nucleotide sequence is determined. One cDNA clone is identified. The cDNA sequence is shown in ***SEQ ID NO:7***. The deduced amino acid sequence is analyzed using the BLASTx search against nucleotide sequence databases (Altschul *et al.* (1990) J Mol. Biol. 215:403-410; Altschul *et al.* (1997) Nucleic Acids Res. 25: 3389-3402). The BLAST search results show that the recovered 3963 cDNA shows sequence similarity to a number of DNA repair proteins, including Rad32p from *Schizosaccharomyces pombe* (Genbank accession numberQ09683); hMre11 from *Homo sapiens* (Genbank accession number U37359); and Mre11p from *Saccharomyces*

cerevisiae (Genbank accession number U60829). Because the genomic DNA that contains the 3963 Open Reading Frame (ORF) was not annotated correctly in the prior art with respect to the exon/intron boundaries, the inventors are the first to provide experimental documentation of the correct ORF for the 3963 gene. The prior art indicates these exon/intron boundaries: 35662-35817, 36015-36172, 36315-36405, 36528-36647, 36728-36796, 36865-36956, 37045-37147, 37247-37354, 37476-37538, 37785-37862, 38060-38122, 38211-38271, 38753-38835, 38979-39092, 39468-39766, 39879-40002, 40161-40370. The exon/intron boundaries corresponding to the partial cDNA disclosed herein are: missing 5' end (first known base at 36147), 36147-36172, 36315-36405, 36528-36647, 36728-36796, 36865-36956, 37045-37147, 37247-37354, 37476-37538, 37610-37681, 37785-39092, 39212-39290, 39377-39445, 39532-39776, 39879-40002, 40161-40363, 40478-40508 (stop begins at 40509).

Example 5: Sequence Analysis of Tagged Seedling – Lethal Line #4036 From the T-DNA Mutagenized Population of *Arabidopsis*

The plasmid rescue technique is used to molecularly clone *Arabidopsis* flanking DNA from one or both sides of the T-DNA insertions resulting from T-DNA mutagenesis. Plasmids obtained in this manner are analyzed by restriction enzyme digestion to sort the plasmids into classes based on their digestion pattern. For each class of plasmid clone, the DNA sequence is determined. The resulting sequences are analyzed for the presence of non-T-DNA vector sequences. The plasmids recovered from the plasmid rescue protocol are sequenced using the slp346 primer (5' GCGGACATCTACATTTTTGA 3'; ***SEQ ID NO:11***). Primer slp346 provides information on the flanking sequence immediately adjacent to the left T-DNA border. The plasmid rescue is validated via PCR of template genomic DNA from a heterozygote for the 4036 insertion mutation. The experiment uses a primer anchored in the predicted flanking sequence and the slp328 primer (5' ACCTTAGGCGACTTTTGAAC 3'; ***SEQ ID NO:15***; anchored in the T-DNA insertion). Finding a PCR product of the size expected based on the sequence of the plasmid rescue clone confirms a valid rescue.

The sequence obtained from the above clone is used in a BLASTn search against nucleotide databases (Altschul *et al.* (1990) J Mol. Biol. 215:403-410; Altschul *et al.* (1997) Nucleic Acids Res. 25;3389-3402). The BLAST results show that the plant flanking sequence is 100% identical to published genomic sequence of P1 MQB2, from

chromosome 5 of *Arabidopsis* (Genbank accession # AB009053). The T-DNA insertion occurred at base 31,380 of the annotated P1 clone and interrupts a gene identified as MQB2.6. The protein encoded by the interrupted open reading frame (ORF) shows similarity to 1-deoxy-D-xylulose 5-phosphate reductoisomerase from a number of organisms including *Synechocystis* sp. (SWISS-PROTQ55663), *Bacillus subtilis* (SWISS-PROT O31753), and *Escherichia coli* (SWISS-PROT P45568) (Takahashi et al. (1998) Proc. Natl. Acad. Sci. USA, 95: 9879-9884). The genomic region encompassing the ORF is re-annotated with Web GeneMark software (Borodovsky, M. and McIninch J. (1993) Computers & Chemistry, 17: 123-133). Primers are then designed to the 5' and 3' ends of the predicted ORF, and PCR is performed using DNA from the pFL61 *Arabidopsis* cDNA library (Minet et al. (1992) Plant J. 2: 417-422) as the template. The resulting PCR product is TA-ligated and cloned (Original TA Cloning Kit, Invitrogen), and sequenced. Because the genomic DNA that contains the 4036 ORF was not annotated correctly in the prior art with respect to the exon/intron boundaries, the inventors are the first to provide experimental documentation of the correct ORF for the 4036 gene. The prior art indicates these exon/intron boundaries: 33490..33356, 31293..31207, 30971..30846, 30780..30718, 30622..30473, 30345..30288, 30194..30083, 29996..29892, 29805..29684, 29394..29248, 29162..28997. In the sequence of the present invention, base 31928 marks the first base of the cDNA's start codon and base 28996 marks the first base of the cDNA's stop codon. The 3' end of the exon containing the start codon is 31836, and the 5' end of the exon containing the stop codon is 29161. The internal exon/intron boundaries for the cDNA disclosed herein are: 31640.. 31448, 31294..31202, 30965..30843, 30777..30722, 30636..30473, 30355..30287, 30193..30082, 29995..29891, 29804..29684, 29394..29247.

Example 6a Expression of Recombinant 245 Protein in *E. coli*

The coding region of the protein, corresponding to the cDNA clone SEQ ID NO: 1, is subcloned into previously described expression vectors, and transformed into *E. coli* using the manufacturer's conditions. Specific examples include plasmids such as pBluescript (Stratagene, La Jolla, CA), pFLAG (International Biotechnologies, Inc., New Haven, CT), and pTrcHis (Invitrogen, La Jolla, CA). *E. coli* is cultured, and expression of the 245 activity is confirmed. Protein conferring 245 activity is isolated using standard techniques.

Example 6b Expression of Recombinant 5283 Protein in *E. coli*

The coding region of the protein, corresponding to the cDNA clone SEQ ID NO: 3, is subcloned into previously described expression vectors, and transformed into *E. coli* using the manufacturer's conditions. Specific examples include plasmids such as pBluescript (Stratagene, La Jolla, CA), pFLAG (International Biotechnologies, Inc., New Haven, CT), and pTrcHis (Invitrogen, La Jolla, CA). *E. coli* is cultured, and expression of the 5283 activity is confirmed. Protein conferring 5283 activity is isolated using standard techniques.

Example 6c Expression of Recombinant 2490 Protein in *E. coli*

The coding region of the protein, corresponding to the cDNA clone SEQ ID NO: 5, is subcloned into previously described expression vectors, and transformed into *E. coli* using the manufacturer's conditions. Specific examples include plasmids such as pBluescript (Stratagene, La Jolla, CA), pFLAG (International Biotechnologies, Inc., New Haven, CT), and pTrcHis (Invitrogen, La Jolla, CA). *E. coli* is cultured, and expression of the 2490 activity is confirmed. Protein conferring 2490 activity is isolated using standard techniques.

Example 6d Expression of Recombinant 3963 Protein in *E. coli*

The coding region of the protein, corresponding to the cDNA clone SEQ ID NO: 7, is subcloned into previously described expression vectors, and transformed into *E. coli* using the manufacturer's conditions. Specific examples include plasmids such as pBluescript (Stratagene, La Jolla, CA), pFLAG (International Biotechnologies, Inc., New Haven, CT), and pTrcHis (Invitrogen, La Jolla, CA). *E. coli* is cultured, and expression of the 3963 activity is confirmed. Protein conferring 3963 activity is isolated using standard techniques.

Example 6e Expression of Recombinant 4036 Protein in *E. coli*

The coding region of the protein, corresponding to the cDNA clone SEQ ID NO: 9, is subcloned into previously described expression vectors, and transformed into *E. coli* using the manufacturer's conditions. Specific examples include plasmids such as pBluescript (Stratagene, La Jolla, CA), pFLAG (International Biotechnologies, Inc., New Haven, CT),

and pTrcHis (Invitrogen, La Jolla, CA). *E. coli* is cultured, and expression of the 4036 activity is confirmed. Protein conferring 4036 activity is isolated using standard techniques.

Example 7: In vitro Recombination of 245, 5283, 2490, 3963, or 4036 Genes by DNA Shuffling

The nucleotide sequence of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9, respectively, is amplified by PCR. The resulting DNA fragment is digested by DNaseI treatment essentially as described (Stemmer et al. (1994) PNAS 91: 10747-10751) and the PCR primers are removed from the reaction mixture. A PCR reaction is carried out without primers and is followed by a PCR reaction with the primers, both as described (Stemmer et al. (1994) PNAS 91: 10747-10751). The resulting DNA fragments are cloned into pTRC99a (Pharmacia, Cat no: 27-5007-01) for use in bacteria, or into pESC vectors (Stratagene Catalog) for use in yeast; and transformed into a bacterial or yeast strain deficient in 245, 5283, 2490, 3963, or 4036 activity, respectively, by electroporation using the Biorad Gene Pulser and the manufacturer's conditions. The transformed bacteria or yeast are grown on medium that contains inhibitory concentrations of an inhibitor of 245, 5283, 2490, 3963, or 4036 activity and those colonies that grow in the presence of the inhibitor are selected. Colonies that grow in the presence of normally inhibitory concentrations of inhibitor are picked and purified by repeated restreaking. Their plasmids are purified and the DNA sequences of cDNA inserts from plasmids that pass this test are then determined.

In a similar reaction, PCR-amplified DNA fragments comprising the *A. thaliana* 245, 5283, 2490, 3963, or 4036 gene, respectively, encoding the protein and PCR-amplified DNA fragments comprising the 245, 5283, 2490, 3963, or 4036 gene, respectively, from *E. coli* are recombined *in vitro* and resulting variants with improved tolerance to the inhibitor are recovered as described above.

Example 8a: In vitro Recombination of 245 Genes by Staggered Extension Process

The *A. thaliana* 245 gene encoding the 245 protein and the *E.coli* 245 gene are each cloned into the polylinker of a pBluescript vector. A PCR reaction is carried out essentially as described (Zhao et al. (1998) Nature Biotechnology 16: 258-261) using the "reverse primer" and the "M13 -20 primer" (Stratagene Catalog). Amplified PCR fragments are digested with appropriate restriction enzymes and cloned into pTRC99a and mutated 245 genes are screened as described in Example 7.

Example 8b: In vitro Recombination of 5283 Genes by Staggered Extension Process

The *A. thaliana* 5283 gene encoding the 5283 protein and the *E.coli* 5283 gene are each cloned into the polylinker of a pBluescript vector. A PCR reaction is carried out essentially as described (Zhao et al. (1998) Nature Biotechnology 16: 258-261) using the "reverse primer" and the "M13 -20 primer" (Stratagene Catalog). Amplified PCR fragments are digested with appropriate restriction enzymes and cloned into pTRC99a and mutated 5283 genes are screened as described in Example 7.

Example 8c: In vitro Recombination of 2490 Genes by Staggered Extension Process

The *A. thaliana* 2490 gene encoding the 2490 protein and the *E.coli* 2490 gene are each cloned into the polylinker of a pBluescript vector. A PCR reaction is carried out essentially as described (Zhao et al. (1998) Nature Biotechnology 16: 258-261) using the "reverse primer" and the "M13 -20 primer" (Stratagene Catalog). Amplified PCR fragments are digested with appropriate restriction enzymes and cloned into pTRC99a and mutated 2490 genes are screened as described in Example 7.

Example 8d: In vitro Recombination of 3963 Genes by Staggered Extension Process

The *A. thaliana* 3963 gene encoding the 3963 protein and the *E.coli* 3963 gene are each cloned into the polylinker of a pBluescript vector. A PCR reaction is carried out essentially as described (Zhao et al. (1998) Nature Biotechnology 16: 258-261) using the "reverse primer" and the "M13 -20 primer" (Stratagene Catalog). Amplified PCR fragments are digested with appropriate restriction enzymes and cloned into pTRC99a and mutated 3963 genes are screened as described in Example 7.

Example 8e: In vitro Recombination of 4036 Genes by Staggered Extension Process

The *A. thaliana* 4036 gene encoding the 4036 protein and the *E.coli* 4036 gene are each cloned into the polylinker of a pBluescript vector. A PCR reaction is carried out essentially as described (Zhao et al. (1998) Nature Biotechnology 16: 258-261) using the "reverse primer" and the "M13 -20 primer" (Stratagene Catalog). Amplified PCR fragments are digested with appropriate restriction enzymes and cloned into pTRC99a and mutated 4036 genes are screened as described in Example 7.

Example 9: In Vitro Binding Assays

Recombinant 245, 5283, 2490, 3963, or 4036 protein is obtained, for example, according to Example 6a,6b,6c,6d,or 6e, respectively. The protein is immobilized on chips appropriate for ligand binding assays using techniques which are well known in the art. The protein immobilized on the chip is exposed to sample compound in solution according to methods well know in the art. While the sample compound is in contact with the immobilized protein measurements capable of detecting protein-ligand interactions are conducted. Examples of such measurements are SELDI, biacore and FCS, described above. Compounds found to bind the protein are readily discovered in this fashion and are subjected to further characterization.

The above disclosed embodiments are illustrative. This disclosure of the invention will place one skilled in the art in possession of many variations of the invention. All such obvious and foreseeable variations are intended to be encompassed by the appended claims.

What Is Claimed Is:

1. An isolated DNA molecule comprising a nucleotide sequence substantially similar to any one of the sequences selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9.
2. The DNA molecule of claim 1, wherein the sequence encodes an amino acid sequence substantially similar to any one of the sequences selected from the group consisting of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10.
3. The DNA molecule of claim 1, wherein the sequence is any one of the sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9.
4. The DNA molecule of claim 1, wherein the sequence encodes the amino acid sequence of any one of the sequences selected from the group consisting of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10.
5. The DNA molecule according to claim 1, wherein said nucleotide sequence is a plant nucleotide sequence.
6. The DNA molecule of claim 5, wherein the plant *is Arabidopsis thaliana.*
7. The DNA molecule of claim 1, wherein the protein has any one of the activities selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity.
8. An amino acid sequence comprising an amino acid sequence encoded by a nucleotide sequence substantially similar to any one of the sequences selected from the group

consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9.

9. The amino acid sequence of claim 8 comprising an amino acid sequence encoded by any one of the sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9.

10. An amino acid sequence comprising an amino acid sequence substantially similar to any one of the sequences selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10.

11. The amino acid sequence of claim 10, wherein the sequence is any one of the sequences selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10.

12. The amino acid sequence of claim 8, wherein the protein has any one of the activities selected from the group of 245, 5283, 2490, 3963 and 4036 activity.

13. An amino acid sequence comprising at least 20 consecutive amino acid residues of the amino acid sequence encoded by any one of the sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9.

14. An amino acid sequence comprising at least 20 consecutive amino acid residues of the amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10.

15. An expression cassette comprising a promoter operatively linked to a DNA molecule according to claim 1.

16. A recombinant vector comprising an expression cassette according to claim 15, wherein said vector is capable of being stably transformed into a host cell.

17. A host cell comprising an expression cassette according to claim 15, wherein said nucleotide sequence is expressible in said cell.

18. A host cell according to claim 17, wherein said host cell is an eukaryotic cell.

19. A host cell according to claim 17, wherein said host cell is selected from the group consisting of an insect cell, a yeast cell, and a plant cell.

20. A host cell according to claim 17, wherein said host cell is a prokaryotic cell.

21. A host cell according to claim 17, wherein said host cell is a bacterial cell.

22. A plant or seed comprising a plant cell of claim 19.

23. A plant of claim 22, wherein said plant is tolerant to an inhibitor of any one of the activities selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity.

24. A process for making nucleotides sequences encoding gene products having altered activity selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity comprising,

a) shuffling a nucleotide sequence of claim 1,

b) expressing the resulting shuffled nucleotide sequences and

c) selecting for altered activity selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity as compared to the activity selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity of the gene product of said unmodified nucleotide sequence.

25. The process of claim 24, wherein the nucleotide sequence is any one of the sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9.

26. A shuffled DNA molecule obtainable by the process of claim 24.

27. A shuffled DNA molecule produced by the process of claim 24.

28. A shuffled DNA molecule obtained by the process of claim 24, wherein said shuffled DNA molecule encodes a gene product having enhanced tolerance to an inhibitor of any one of the activities selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity.

29. An expression cassette comprising a promoter operatively linked to a nucleotide sequence according to claim 26.

30. A recombinant vector comprising an expression cassette according to claim 29, wherein said vector is capable of being stably transformed into a host cell.

31. A host cell comprising an expression cassette according to claim 29, wherein said nucleotide sequence is expressible in said cell.

32. A host cell according to claim 31, wherein said host cell is an eukaryotic cell.

33. A host cell according to claim 31, wherein said host cell is selected from the group consisting of an insect cell, a yeast cell, and a plant cell.

34. A host cell according to claim 31, wherein said host cell is a prokaryotic cell.

35. A host cell according to claim 31, wherein said host cell is a bacterial cell.

36. A plant or seed comprising a plant cell of claim 33.

37. A plant of claim 36, wherein said plant is tolerant to an inhibitor selected from the group consisting of 245, 5283, 2490, 3963 and 4036 activity.

38. A method for selecting compounds that interact with the protein encoded by any one of the sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ

ID NO:5, SEQ ID NO:7 and SEQ ID NO:9. ????

, comprising:

a) expressing a DNA molecule comprising any one of the sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9 or a sequence substantially similar to any one of the sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9to generate the corresponding protein,

b) testing a compound suspected of having the ability to interact with the protein expressed in step (a), and

c) selecting compounds that interact with the protein in step (b).

39. A process of identifying an inhibitor of any one of the activities selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity comprising:

a) introducing a DNA molecule comprising a nucleotide sequence of any one of the sequences selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9 and having any one of the activities selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity, or nucleotide sequences substantially similar thereto, or a homolog thereof, into a plant cell, such that said sequence is functionally expressible at levels that are higher than wild-type expression levels,

b) combining said plant cell with a compound to be tested for the ability to inhibit any one of the activities selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity under conditions conducive to such inhibition,

c) measuring plant cell growth under the conditions of step (b), and

d) comparing the growth of said plant cell with the growth of a plant cell having an unaltered activity selected from the group consisting of 245 activity, 5283 activity, 2490 activity, 3963 activity and 4036 activity under identical conditions, and

e) selecting said compound that inhibits plant cell growth in step (d).

40. A compound having herbicidal activity identifiable according to the process of claim 39.

41. A process of identifying compounds having herbicidal activity comprising:

a) combining a protein of claim 8 and a compound to be tested for the ability to interact with said protein, under conditions conducive to interaction,

b) selecting a compound identified in step (a) that is capable of interacting with said protein,

c) applying identified compound in step (b) to a plant to test for herbicidal activity, and

d) selecting compounds having herbicidal activity.

42. A compound having herbicidal activity identifiable according to the process of claim 41.

43. A method for suppressing the growth of a plant comprising, applying to said plant a compound that inhibits the activity of the amino acid sequence of claim 8 in an amount sufficient to suppress the growth of said plant.

44. The method of claim 41, wherein the compound is a compound having herbicidal activity identifiable according to the process of claim 39.

45. A method of improving crops comprising, applying to a herbicide tolerant plant or seed selected from the group consisting of the plant or seed of claim 23 and the plant or seed of claim 37, a compound having herbicidal activity identifiable according to a process selected from the group of the method of claim 38, the process of claim 39, and the process of claim 41, in an amount that inhibits the growth of undesired vegetation without significantly suppressing the growth of the herbicide tolerant plant or seed.

46. A DNA molecule comprising a nucleotide sequence substantially similar to any one of the sequences selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29.

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tca ttt aag gct ttc gaa gag ata tgt tct ata gct gag gag aaa cag 144
Ser Phe Lys Ala Phe Glu Glu Ile Cys Ser Ile Ala Glu Glu Lys Gln
35 40 45

gtg gac ttc tta ctc ctc gga ggt gat ctt ttt cat gag aat aaa ccc 192
Val Asp Phe Leu Leu Leu Gly Gly Asp Leu Phe His Glu Asn Lys Pro
50 55 60

tct aga act acg tta gtt aaa gcc att gaa att ctt cgt cgc cac tgt 240
Ser Arg Thr Thr Leu Val Lys Ala Ile Glu Ile Leu Arg Arg His Cys
65 70 75 80

ctg aat gat aaa cca gtg cag ttt caa gta gtc agc gac cag aca gta 288
Leu Asn Asp Lys Pro Val Gln Phe Gln Val Val Ser Asp Gln Thr Val

85 90 95

aat ttt cag aat gcg ttt ggt caa gtc aat tac gag gat cca cac ttc 336
Asn Phe Gln Asn Ala Phe Gly Gln Val Asn Tyr Glu Asp Pro His Phe
100 105 110

aat gta ggc ttg ccc gtg ttc agt att cat gga aac cat gat gat cca 384
Asn Val Gly Leu Pro Val Phe Ser Ile His Gly Asn His Asp Asp Pro
115 120 125

gcc gga gtg gac aat ctt tct gca att gat att ctt tcc gca tgc aac 432
Ala Gly Val Asp Asn Leu Ser Ala Ile Asp Ile Leu Ser Ala Cys Asn
130 135 140

ctt gtg aac tat ttt gga aag atg gtt ctt ggt ggt tct ggt gtt ggc 480
Leu Val Asn Tyr Phe Gly Lys Met Val Leu Gly Gly Ser Gly Val Gly
145 150 155 160

cag att act ctc tac cct ata ctt atg aag aag ggc tca aca acc gtg 528
Gln Ile Thr Leu Tyr Pro Ile Leu Met Lys Lys Gly Ser Thr Thr Val
165 170 175

gct ctc tat ggt tta gga aac atc agg gat gaa cgt ctc aat aga atg 576
Ala Leu Tyr Gly Leu Gly Asn Ile Arg Asp Glu Arg Leu Asn Arg Met
180 185 190

ttt cag acc cca cat gct gtc caa tgg atg agg cct gaa gtt caa gaa 624
Phe Gln Thr Pro His Ala Val Gln Trp Met Arg Pro Glu Val Gln Glu
195 200 205

gga tgt gat gtt tct gac tgg ttc aac att ctg gtg ctt cat caa aat 672
Gly Cys Asp Val Ser Asp Trp Phe Asn Ile Leu Val Leu His Gln Asn
210 215 220

agg gtg aaa tca aac ccc aaa aat gca ata agt gag cac ttt ctt cca 720
Arg Val Lys Ser Asn Pro Lys Asn Ala Ile Ser Glu His Phe Leu Pro
225 230 235 240

cgt ttc ctc gac ttc att gtg tgg ggc cat gag cat gaa tgc cta atc 768
Arg Phe Leu Asp Phe Ile Val Trp Gly His Glu His Glu Cys Leu Ile
245 250 255

gac ccc cag gag gta tct gga atg ggc ttc cac atc aca caa cca gga 816
Asp Pro Gln Glu Val Ser Gly Met Gly Phe His Ile Thr Gln Pro Gly
260 265 270

tct tct gtg gca aca tca ctt att gat ggg gaa tcg aag cca aaa cat 864
Ser Ser Val Ala Thr Ser Leu Ile Asp Gly Glu Ser Lys Pro Lys His
275 280 285

gtt ctt ctc tta gaa atc aag gga aat caa tat cgt cct acg aag ata 912
Val Leu Leu Leu Glu Ile Lys Gly Asn Gln Tyr Arg Pro Thr Lys Ile
290 295 300

cct ttg aca tct gtg agg cct ttt gag tat aca gag att gtt tta aag 960

Pro Leu Thr Ser Val Arg Pro Phe Glu Tyr Thr Glu Ile Val Leu Lys
305 310 315 320

gat gaa agt gat att gat ccc aat gat caa aac tca att ctg gaa cac 1008
Asp Glu Ser Asp Ile Asp Pro Asn Asp Gln Asn Ser Ile Leu Glu His
325 330 335

ttg gat aaa gtg gtc aga aat cta ata gag aaa gct agc aaa aaa gct 1056
Leu Asp Lys Val Val Arg Asn Leu Ile Glu Lys Ala Ser Lys Lys Ala
340 345 350

gtt aac aga tca gag atc aaa ctc cca ttg gtt cga atc aag gta gat 1104
Val Asn Arg Ser Glu Ile Lys Leu Pro Leu Val Arg Ile Lys Val Asp
355 360 365

tat tct gga ttt atg acg ata aat cct caa aga ttt gga cag aaa tat 1152
Tyr Ser Gly Phe Met Thr Ile Asn Pro Gln Arg Phe Gly Gln Lys Tyr
370 375 380

gtg gga aag gtt gca aat ccc cag gac att ttg ata ttt tcc aag gct 1200
Val Gly Lys Val Ala Asn Pro Gln Asp Ile Leu Ile Phe Ser Lys Ala
385 390 395 400

tct aag aag ggt cgg agc gaa gcc aac atc gat gat tct gag cgg ctt 1248
Ser Lys Lys Gly Arg Ser Glu Ala Asn Ile Asp Asp Ser Glu Arg Leu
405 410 415

cgt cca gaa gaa ctg aac cag cag aat ata gaa gct tta gta gct gaa 1296
Arg Pro Glu Glu Leu Asn Gln Gln Asn Ile Glu Ala Leu Val Ala Glu
420 425 430

agc aac ctg aaa atg gag atc ctt cca gtt aac gat ctg gat gtt gct 1344
Ser Asn Leu Lys Met Glu Ile Leu Pro Val Asn Asp Leu Asp Val Ala
435 440 445

ctt cac aat ttt gtg aac aag gat gat aaa cta gcc ttc tac tca tgc 1392
Leu His Asn Phe Val Asn Lys Asp Asp Lys Leu Ala Phe Tyr Ser Cys
450 455 460

gtt cag tac aat ctt caa gag act cgt ggt aaa ctt gca aag gat tca 1440
Val Gln Tyr Asn Leu Gln Glu Thr Arg Gly Lys Leu Ala Lys Asp Ser
465 470 475 480

gat gcc aag aaa ttt gag gaa gat gac ttg att ctt aaa gtg gga gag 1488
Asp Ala Lys Lys Phe Glu Glu Asp Asp Leu Ile Leu Lys Val Gly Glu
485 490 495

tgc tta gag gaa cgc ttg aaa gat agg tcc act cga ccc act ggt tcc 1536
Cys Leu Glu Glu Arg Leu Lys Asp Arg Ser Thr Arg Pro Thr Gly Ser
500 505 510

tca cag ttt tta tcc act gga ttg act tca gag aat ttg aca aaa gga 1584
Ser Gln Phe Leu Ser Thr Gly Leu Thr Ser Glu Asn Leu Thr Lys Gly
515 520 525

agc agt ggc atc gcg aat gct tcg ttc agt gat gat gaa gac aca act 1632
Ser Ser Gly Ile Ala Asn Ala Ser Phe Ser Asp Asp Glu Asp Thr Thr
530 535 540

cag atg tct ggt tta gct cct ccc act aga gga cga aga ggt tca tcc 1680
Gln Met Ser Gly Leu Ala Pro Pro Thr Arg Gly Arg Arg Gly Ser Ser
545 550 555 560

act gct aat aca act cgt ggt aga gct aaa gcc cca acc aga gga cga 1728
Thr Ala Asn Thr Thr Arg Gly Arg Ala Lys Ala Pro Thr Arg Gly Arg
565 570 575

ggc cgt ggt aag gcc tca agt gcg atg aag caa acc act ctt gat agt 1776
Gly Arg Gly Lys Ala Ser Ser Ala Met Lys Gln Thr Thr Leu Asp Ser
580 585 590

tct ctt ggt ttc cgc cag tct caa aga tct gct tcg gct gct gct tca 1824
Ser Leu Gly Phe Arg Gln Ser Gln Arg Ser Ala Ser Ala Ala Ala Ser
595 600 605

gct gcc ttc aaa agt gct tcc acc att gga gaa gat gat gta gat tct 1872
Ala Ala Phe Lys Ser Ala Ser Thr Ile Gly Glu Asp Asp Val Asp Ser
610 615 620

cct tca agc gaa gaa gtc gag cct gaa gat ttt aac aaa cct gac agc 1920
Pro Ser Ser Glu Glu Val Glu Pro Glu Asp Phe Asn Lys Pro Asp Ser
625 630 635 640

agt tcg gag gac gat gag agc act aaa ggc aaa gga cgt aaa aga cca 1968
Ser Ser Glu Asp Asp Glu Ser Thr Lys Gly Lys Gly Arg Lys Arg Pro
645 650 655

gct act act aag aga ggc aga ggt aga ggt tct ggg act tca aaa cgt 2016
Ala Thr Thr Lys Arg Gly Arg Gly Arg Gly Ser Gly Thr Ser Lys Arg
660 665 670

ggt aga aaa aac gaa agc tct tct tca ctt aat agg cta ctc agt agc 2064
Gly Arg Lys Asn Glu Ser Ser Ser Ser Leu Asn Arg Leu Leu Ser Ser
675 680 685

aaa gac gat gac gag gac gaa gat gat gaa gac aga gaa aag aag ctt 2112
Lys Asp Asp Asp Glu Asp Glu Asp Asp Glu Asp Arg Glu Lys Lys Leu
690 695 700

aac aaa tct cag cct cgg gtt aca agg aac tat gga gct cta aga aga 2160
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taa 2163

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<213> Arabidopsis thaliana

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        35                  40                  45
Val Asp Phe Leu Leu Leu Gly Gly Asp Leu Phe His Glu Asn Lys Pro
    50                  55                  60
Ser Arg Thr Thr Leu Val Lys Ala Ile Glu Ile Leu Arg Arg His Cys
65                  70                  75                  80
Leu Asn Asp Lys Pro Val Gln Phe Gln Val Val Ser Asp Gln Thr Val
                85                  90                  95
Asn Phe Gln Asn Ala Phe Gly Gln Val Asn Tyr Glu Asp Pro His Phe
            100                 105                 110
Asn Val Gly Leu Pro Val Phe Ser Ile His Gly Asn His Asp Asp Pro
        115                 120                 125
Ala Gly Val Asp Asn Leu Ser Ala Ile Asp Ile Leu Ser Ala Cys Asn
    130                 135                 140
Leu Val Asn Tyr Phe Gly Lys Met Val Leu Gly Gly Ser Gly Val Gly
145                 150                 155                 160
Gln Ile Thr Leu Tyr Pro Ile Leu Met Lys Lys Gly Ser Thr Thr Val
                165                 170                 175
Ala Leu Tyr Gly Leu Gly Asn Ile Arg Asp Glu Arg Leu Asn Arg Met
            180                 185                 190
Phe Gln Thr Pro His Ala Val Gln Trp Met Arg Pro Glu Val Gln Glu
        195                 200                 205
Gly Cys Asp Val Ser Asp Trp Phe Asn Ile Leu Val Leu His Gln Asn
    210                 215                 220
Arg Val Lys Ser Asn Pro Lys Asn Ala Ile Ser Glu His Phe Leu Pro
225                 230                 235                 240
Arg Phe Leu Asp Phe Ile Val Trp Gly His Glu His Glu Cys Leu Ile
                245                 250                 255
Asp Pro Gln Glu Val Ser Gly Met Gly Phe His Ile Thr Gln Pro Gly
            260                 265                 270
Ser Ser Val Ala Thr Ser Leu Ile Asp Gly Glu Ser Lys Pro Lys His
        275                 280                 285
Val Leu Leu Leu Glu Ile Lys Gly Asn Gln Tyr Arg Pro Thr Lys Ile
    290                 295                 300
Pro Leu Thr Ser Val Arg Pro Phe Glu Tyr Thr Glu Ile Val Leu Lys
305                 310                 315                 320
Asp Glu Ser Asp Ile Asp Pro Asn Asp Gln Asn Ser Ile Leu Glu His
                325                 330                 335
Leu Asp Lys Val Val Arg Asn Leu Ile Glu Lys Ala Ser Lys Lys Ala
            340                 345                 350
Val Asn Arg Ser Glu Ile Lys Leu Pro Leu Val Arg Ile Lys Val Asp
        355                 360                 365
Tyr Ser Gly Phe Met Thr Ile Asn Pro Gln Arg Phe Gly Gln Lys Tyr
    370                 375                 380
Val Gly Lys Val Ala Asn Pro Gln Asp Ile Leu Ile Phe Ser Lys Ala
385                 390                 395                 400
Ser Lys Lys Gly Arg Ser Glu Ala Asn Ile Asp Asp Ser Glu Arg Leu
                405                 410                 415
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Arg Pro Glu Glu Leu Asn Gln Gln Asn Ile Glu Ala Leu Val Ala Glu
420 425 430
Ser Asn Leu Lys Met Glu Ile Leu Pro Val Asn Asp Leu Asp Val Ala
435 440 445
Leu His Asn Phe Val Asn Lys Asp Asp Lys Leu Ala Phe Tyr Ser Cys
450 455 460
Val Gln Tyr Asn Leu Gln Glu Thr Arg Gly Lys Leu Ala Lys Asp Ser
465 470 475 480
Asp Ala Lys Lys Phe Glu Glu Asp Asp Leu Ile Leu Lys Val Gly Glu
485 490 495
Cys Leu Glu Glu Arg Leu Lys Asp Arg Ser Thr Arg Pro Thr Gly Ser
500 505 510
Ser Gln Phe Leu Ser Thr Gly Leu Thr Ser Glu Asn Leu Thr Lys Gly
515 520 525
Ser Ser Gly Ile Ala Asn Ala Ser Phe Ser Asp Asp Glu Asp Thr Thr
530 535 540
Gln Met Ser Gly Leu Ala Pro Pro Thr Arg Gly Arg Arg Gly Ser Ser
545 550 555 560
Thr Ala Asn Thr Thr Arg Gly Arg Ala Lys Ala Pro Thr Arg Gly Arg
565 570 575
Gly Arg Gly Lys Ala Ser Ser Ala Met Lys Gln Thr Thr Leu Asp Ser
580 585 590
Ser Leu Gly Phe Arg Gln Ser Gln Arg Ser Ala Ser Ala Ala Ala Ser
595 600 605
Ala Ala Phe Lys Ser Ala Ser Thr Ile Gly Glu Asp Asp Val Asp Ser
610 615 620
Pro Ser Ser Glu Glu Val Glu Pro Glu Asp Phe Asn Lys Pro Asp Ser
625 630 635 640
Ser Ser Glu Asp Asp Glu Ser Thr Lys Gly Lys Gly Arg Lys Arg Pro
645 650 655
Ala Thr Thr Lys Arg Gly Arg Gly Arg Gly Ser Gly Thr Ser Lys Arg
660 665 670
Gly Arg Lys Asn Glu Ser Ser Ser Ser Leu Asn Arg Leu Leu Ser Ser
675 680 685
Lys Asp Asp Asp Glu Asp Glu Asp Asp Glu Asp Arg Glu Lys Lys Leu
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705 710 715 720

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ttc ttg gat acc tcc agg ttc aat cca atc cct aaa ctc tca ggt ggg      96
Phe Leu Asp Thr Ser Arg Phe Asn Pro Ile Pro Lys Leu Ser Gly Gly
            20                  25                  30

ttt agt ttg agg agg agg gat caa ggg aga ggt ttt gga aaa ggt gtt     144
Phe Ser Leu Arg Arg Arg Asp Gln Gly Arg Gly Phe Gly Lys Gly Val
        35                  40                  45

aag tgt tca gtg aaa gtg cag cag caa caa caa cct cct cca gca tgg     192
Lys Cys Ser Val Lys Val Gln Gln Gln Gln Gln Pro Pro Pro Ala Trp
    50                  55                  60

cct ggg aga gct gtt cct gag gcg cct cgt caa tct tgg gat gga cca     240
Pro Gly Arg Ala Val Pro Glu Ala Pro Arg Gln Ser Trp Asp Gly Pro
65                  70                  75                  80

aaa ccc atc tct atc gtt gga tct act ggt tcc atc ggc act cag aca     288
Lys Pro Ile Ser Ile Val Gly Ser Thr Gly Ser Ile Gly Thr Gln Thr
                85                  90                  95

ttg gat att gtg gct gag aat cct gac aaa ttt aga gtt gtg gct cta     336
Leu Asp Ile Val Ala Glu Asn Pro Asp Lys Phe Arg Val Val Ala Leu
            100                 105                 110

gct gct ggt tcg aat gtt act cta ctt gct gat cag gta agg aga ttt     384
Ala Ala Gly Ser Asn Val Thr Leu Leu Ala Asp Gln Val Arg Arg Phe
        115                 120                 125

aag cct gcg ttg gtt gct gtt aga aac gag tca ctg att aat gag ctt     432
Lys Pro Ala Leu Val Ala Val Arg Asn Glu Ser Leu Ile Asn Glu Leu
    130                 135                 140

aaa gag gct tta gct gat ttg gac tat aaa ccc gag att att cca gga     480
Lys Glu Ala Leu Ala Asp Leu Asp Tyr Lys Pro Glu Ile Ile Pro Gly
145                 150                 155                 160

gag cta gga gtg att gag gtt gcc cga cat cct gaa gct gta acc gtt     528
Glu Leu Gly Val Ile Glu Val Ala Arg His Pro Glu Ala Val Thr Val
                165                 170                 175

gtt acc gga ata gta ggt tgt gcg gga ctg aag cct acg gtt gct gca     576
Val Thr Gly Ile Val Gly Cys Ala Gly Leu Lys Pro Thr Val Ala Ala
            180                 185                 190

att gaa gca gga aag gac att gct ctt gca aac aaa gag aca tta atc     624
Ile Glu Ala Gly Lys Asp Ile Ala Leu Ala Asn Lys Glu Thr Leu Ile
        195                 200                 205

gca ggt ggt cct ttc gtg ctt ccg ctt gcc aac aaa cat aat gta aag     672
Ala Gly Gly Pro Phe Val Leu Pro Leu Ala Asn Lys His Asn Val Lys
    210                 215                 220

att ctt ccg gca gat tca gaa cat tct gcc ata ttt cag tgt att caa     720
Ile Leu Pro Ala Asp Ser Glu His Ser Ala Ile Phe Gln Cys Ile Gln
225                 230                 235                 240
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ggt ttg cct gaa ggc gct ctg cgc aag ata atc ttg act gca tct ggt 768
Gly Leu Pro Glu Gly Ala Leu Arg Lys Ile Ile Leu Thr Ala Ser Gly
245 250 255

gga gct ttt agg gat tgg cct gtc gaa aag cta aag gaa gtt aaa gta 816
Gly Ala Phe Arg Asp Trp Pro Val Glu Lys Leu Lys Glu Val Lys Val
260 265 270

gcg gat gcg ttg aag cat cca aac tgg aac atg gga aag aaa atc act 864
Ala Asp Ala Leu Lys His Pro Asn Trp Asn Met Gly Lys Lys Ile Thr
275 280 285

gtg gac tct gct acg ctt ttc aac aag ggt ctt gag gtc att gaa gcg 912
Val Asp Ser Ala Thr Leu Phe Asn Lys Gly Leu Glu Val Ile Glu Ala
290 295 300

cat tat ttg ttt gga gct gag tat gac gat ata gag att gtc att cat 960
His Tyr Leu Phe Gly Ala Glu Tyr Asp Asp Ile Glu Ile Val Ile His
305 310 315 320

cct caa agt atc ata cat tcc atg att gaa aca cag gat tca tct gtg 1008
Pro Gln Ser Ile Ile His Ser Met Ile Glu Thr Gln Asp Ser Ser Val
325 330 335

ctt gct caa ttg ggt tgg cct gat atg cgt tta ccg att ctc tac acc 1056
Leu Ala Gln Leu Gly Trp Pro Asp Met Arg Leu Pro Ile Leu Tyr Thr
340 345 350

atg tca tgg ccc gat aga gtt cct tgt tct gaa gta act tgg cct aga 1104
Met Ser Trp Pro Asp Arg Val Pro Cys Ser Glu Val Thr Trp Pro Arg
355 360 365

ctt gac ctt tgc aaa ctc ggt tca ttg act ttc aag aaa cca gac aat 1152
Leu Asp Leu Cys Lys Leu Gly Ser Leu Thr Phe Lys Lys Pro Asp Asn
370 375 380

gtg aaa tac cca tcc atg gat ctt gct tat gct gct gga cga gct gga 1200
Val Lys Tyr Pro Ser Met Asp Leu Ala Tyr Ala Ala Gly Arg Ala Gly
385 390 395 400

ggc aca atg act gga gtt ctc agc gcc gcc aat gag aaa gct gtt gaa 1248
Gly Thr Met Thr Gly Val Leu Ser Ala Ala Asn Glu Lys Ala Val Glu
405 410 415

atg ttt att gat gaa aag ata agc tat ttg gat atc ttc aag gtt gtg 1296
Met Phe Ile Asp Glu Lys Ile Ser Tyr Leu Asp Ile Phe Lys Val Val
420 425 430

gaa tta aca tgc gat aaa cat cga aac gag ttg gta aca tca ccg tct 1344
Glu Leu Thr Cys Asp Lys His Arg Asn Glu Leu Val Thr Ser Pro Ser
435 440 445

ctt gaa gag att gtt cac tat gac ttg tgg gca cgt gaa tat gcc gcg 1392
Leu Glu Glu Ile Val His Tyr Asp Leu Trp Ala Arg Glu Tyr Ala Ala

450 455 460

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<213> Arabidopsis thaliana

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35 40 45
Lys Cys Ser Val Lys Val Gln Gln Gln Gln Gln Pro Pro Pro Ala Trp
50 55 60
Pro Gly Arg Ala Val Pro Glu Ala Pro Arg Gln Ser Trp Asp Gly Pro
65 70 75 80
Lys Pro Ile Ser Ile Val Gly Ser Thr Gly Ser Ile Gly Thr Gln Thr
85 90 95
Leu Asp Ile Val Ala Glu Asn Pro Asp Lys Phe Arg Val Val Ala Leu
100 105 110
Ala Ala Gly Ser Asn Val Thr Leu Leu Ala Asp Gln Val Arg Arg Phe
115 120 125
Lys Pro Ala Leu Val Ala Val Arg Asn Glu Ser Leu Ile Asn Glu Leu
130 135 140
Lys Glu Ala Leu Ala Asp Leu Asp Tyr Lys Pro Glu Ile Ile Pro Gly
145 150 155 160
Glu Leu Gly Val Ile Glu Val Ala Arg His Pro Glu Ala Val Thr Val
165 170 175
Val Thr Gly Ile Val Gly Cys Ala Gly Leu Lys Pro Thr Val Ala Ala
180 185 190
Ile Glu Ala Gly Lys Asp Ile Ala Leu Ala Asn Lys Glu Thr Leu Ile
195 200 205
Ala Gly Gly Pro Phe Val Leu Pro Leu Ala Asn Lys His Asn Val Lys
210 215 220
Ile Leu Pro Ala Asp Ser Glu His Ser Ala Ile Phe Gln Cys Ile Gln
225 230 235 240
Gly Leu Pro Glu Gly Ala Leu Arg Lys Ile Ile Leu Thr Ala Ser Gly
245 250 255
Gly Ala Phe Arg Asp Trp Pro Val Glu Lys Leu Lys Glu Val Lys Val
260 265 270
Ala Asp Ala Leu Lys His Pro Asn Trp Asn Met Gly Lys Lys Ile Thr
275 280 285
Val Asp Ser Ala Thr Leu Phe Asn Lys Gly Leu Glu Val Ile Glu Ala
290 295 300
His Tyr Leu Phe Gly Ala Glu Tyr Asp Asp Ile Glu Ile Val Ile His
305 310 315 320
Pro Gln Ser Ile Ile His Ser Met Ile Glu Thr Gln Asp Ser Ser Val
325 330 335

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Leu Ala Gln Leu Gly Trp Pro Asp Met Arg Leu Pro Ile Leu Tyr Thr
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Leu Asp Leu Cys Lys Leu Gly Ser Leu Thr Phe Lys Lys Pro Asp Asn
    370                 375                 380
Val Lys Tyr Pro Ser Met Asp Leu Ala Tyr Ala Ala Gly Arg Ala Gly
385                 390                 395                 400
Gly Thr Met Thr Gly Val Leu Ser Ala Ala Asn Glu Lys Ala Val Glu
                405                 410                 415
Met Phe Ile Asp Glu Lys Ile Ser Tyr Leu Asp Ile Phe Lys Val Val
            420                 425                 430
Glu Leu Thr Cys Asp Lys His Arg Asn Glu Leu Val Thr Ser Pro Ser
        435                 440                 445
Leu Glu Glu Ile Val His Tyr Asp Leu Trp Ala Arg Glu Tyr Ala Ala
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<210> 12
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atacattata agttataact ctctttctcg taactaatca ctttcgtgtc cattatcatg 480
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ctttggttta ctgcatttta tgcagattat ataacatgag gtttttgatg caggagaaag 780
aaaccggttt gcaaacagga tcaaagctct aaaccggttg aaggcgaagc tacttgtgat 840
agcaaaagag caaaaggttt cggatgtaaa taaaatcgac agcaagaaca ttttggaacc 900
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gcgggaagaa accaggagtt atgtctctaa gggtcacaag atggtggttg atagaaaaac 960
cggtttagag attctggacc tgaaatcggt cttggatgga aacattggac cactccttgg 1020
agctcatatt agcatgagaa gatcaattga tgcgatttag gcttaatcaa ttggtacttt 1080
aattgctttt tgttttgtat ccaaaaagca acaaatggtt gcttgtgtgt gtatatatat 1140
aaccttcttg tccagaacca tatatgattc taaccatcaa acaaagataa gaattggtga 1200
ctatgtgcta tactctacaa tatcaccatg aatacttcaa actagacttt tgataaattt 1260
tgaaacggtt attaccaata aaacgaaaac catgaaactc ttgttttaat tatcagattc 1320
gagaaagttg tgtacaaaca tagctgagaa ggg 1353

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accaagatta gaattggtga ctaaaaaaaa agaaaaaaaa aaaaaaaaaa aaaaaaaaaa 180
aaaa 184

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<211> 2170
<212> DNA
<213> Arabidopsis thaliana

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tttcaaaatt agggattccg agatcataga tattgctttg tttccgagat ttgagttatt 180
cttaagcttg ttttactaac tttcaatatg ttggatttgt tataggcaac tcttgaagat 240
tctttccttg ctgatttgga cgagttatct gacaatgaag cagaattggt gagtgttaaa 300
acacttttga ttactattat ctgtttactt ggaggagcta tgattgtaat tgtagtttgt 360
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<220>
<223> Description of Artificial Sequence:
oligonucleotide

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<210> 16
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<220>
<223> Description of Artificial Sequence:
oligonucleotide

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<210> 17
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<213> Arabidopsis thaliana

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<211> 218
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<213> Arabidopsis thaliana

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<211> 4140
<212> DNA
<213> Arabidopsis thaliana

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<211> 77
<212> DNA
<213> Arabidopsis thaliana

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caaagaaaca attagat 77

<210> 21
<211> 354
<212> DNA
<213> Arabidopsis thaliana

<400> 21
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<210> 22
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 22
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<210> 23
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

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<210> 24
<211> 5077
<212> DNA
<213> Arabidopsis thaliana

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<210> 25
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

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<210> 26
<211> 255
<212> DNA
<213> Arabidopsis thaliana

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<210> 27
<211> 2935
<212> DNA
<213> Arabidopsis thaliana

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20 July 2000 (20.07.2000)

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(21) International Application Number: PCT/EP00/00246

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(25) Filing Language: English

(26) Publication Language: English

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09/252,336	18 February 1999 (18.02.1999)	US
09/281,376	30 March 1999 (30.03.1999)	US

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(74) Agent: BECKER, Konrad; Novartis AG, Patent and Trademark Dept. Agribusiness, Site Rosental, CH-4002 Basel (CH).

(81) Designated States (*national*): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

(88) Date of publication of the international search report: 7 December 2000

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/42205 A3

(54) Title: HERBICIDE TARGET GENE AND METHODS

(57) Abstract: The invention relates to genes isolated from *Arabidopsis* that code for proteins essential for seedling growth. The invention also includes the methods of using these proteins to discover new herbicides, based on the essentiality of the genes for normal growth and development. The invention can also be used in a screening assay to identify inhibitors that are potential herbicides. The invention is also applied to the development of herbicide tolerant plants, plant tissues, plant seeds, and plant cells.

INTERNATIONAL SEARCH REPORT

Intern: ...al Application No
PCT/EP 00/00246

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C12N5/04 C12N15/52 C12N9/00 C07K14/415
A01H1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 162 602 A (SOMERS DAVID A ET AL) 10 November 1992 (1992-11-10) column 4, line 66 -column 6, line 56 ---	1
X	US 5 013 659 A (BEDBROOCK JOHN R ET AL) 7 May 1991 (1991-05-07) column 2, line 55 -column 4, line 2 ---	1
X	EP 0 154 204 A (MOLECULAR GENETICS INC) 11 September 1985 (1985-09-11) page 13, line 30 -page 14, line 30 ---	1
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 June 2000	04. 9. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mateo Rosell, A.M.

2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TOPPING JENNIFER F ET AL: "Mutations in the HYDRA1 gene of Arabidoposis perturb cell shape and disrupt embryonic and seedling morphogenesis." DEVELOPMENT (CAMBRIDGE) NOV., 1997, vol. 124, no. 21, November 1997 (1997-11), pages 4415-4424, XP000920674 ISSN: 0950-1991 the whole document ---	1
X	ZHOU D -X ET AL: "COP1b, an isoform of COP1 generated by alternative splicing, has a negative effect on COP1 function in regulating light-dependent seedling development in Arabidopsis." MOLECULAR & GENERAL GENETICS FEB., 1998, vol. 257, no. 4, February 1998 (1998-02), pages 387-391, XP000920653 ISSN: 0026-8925 the whole document ---	1
X	DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES,13 October 1997 (1997-10-13), XP002140496 HINXTON, GB AC = B24357. F17K7TR IGF Arabidopsis thaliana genomic clone F17K7, genomic survey sequence. abstract ---	1
A	DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES,1 December 1992 (1992-12-01), XP002140497 HINXTON, GB cited in the application AC = P28353. PEPTIDE CHAIN RELEASE FACTOR 2 (RF-2). Salmonella typhimurium. abstract ---	1,2
A	DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES,1 November 1997 (1997-11-01), XP002140498 HINXTON, GB cited in the application AC= 005782. PEPTIDE CHAIN RELEASE FACTOR 2 (RF-2). PRFB OR RV3105C OR MTCY164.15C. Mycobacterium tuberculosis. abstract ---	1,2

-/--

2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/00246

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES,1 April 1998 (1998-04-01), XP002140499 HINXTON, GB cited in the application AC = P07012; P76642. PEPTIDE CHAIN RELEASE FACTOR 2 (RF-2). PRFB OR SUPK. Escherichia coli. abstract ---	1,2
A	DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES,1 February 1997 (1997-02-01), XP002140500 HINXTON, GB cited in the application AC = P74476. PEPTIDE CHAIN RELEASE FACTOR. PFBB. Synechocystis sp. (strain PCC 6803) abstract ---	1,2
A	ITO KOICHI ET AL: "Single amino acid substitution in prokaryote polypeptide release factor 2 permits it to terminate translation at all three stop codons." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA JULY 7, 1998, vol. 95, no. 14, 7 July 1998 (1998-07-07), pages 8165-8169, XP002140501 ISSN: 0027-8424 cited in the application the whole document ---	1,2
A	FELDMANN K A ET AL: "A DWARF MUTANT OF ARABIDOPSIS GENERATED BY T-DNA INSERTION MUTAGENESIS" SCIENCE,US,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 243, 10 March 1989 (1989-03-10), pages 1351-1354, XP002036944 ISSN: 0036-8075 the whole document ---	1
A	KONCZ ET AL: "isolation of a gene encoding a novel chloroplast protein by T-DNA tagging in Arabidopsis thaliana" EMBO JOURNAL,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 5, no. 9, 1 January 1990 (1990-01-01), pages 1337-1346, XP002076478 ISSN: 0261-4189 the whole document ---	1

-/--

2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	AZPIROZ-LEEHAN R ET AL: "T-DNA insertion mutagenesis in Arabidopsis: going back and forth" TRENDS IN GENETICS, NL, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, vol. 13, no. 4, 1 April 1997 (1997-04-01), pages 152-156, XP004056902 ISSN: 0168-9525 the whole document ---	
E	WO 00 15809 A (NOVARTIS ERFINDUNGEN VERWALTUN ;NOVARTIS AG (CH); BUDZISZEWSKI GRE) 23 March 2000 (2000-03-23) the whole document -----	1-46

2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/00246

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-46 partially

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-46 partially

An isolated DNA molecule comprising a nucleotide sequence substantially similar to SEQ.ID.N.1, wherein the sequence encodes an amino acid sequence similar to SEQ.ID.N.2 which is isolated from a plant and has "245" activity; an expression cassette; a recombinant vector; a host cell; a transformed plant; a method for obtaining shuffled sequences from SEQ.ID.N.1; a method for selecting compounds interacting with the encoded protein; inhibitors of "245" activity; a process of identifying compounds having herbicidal activity.

2. Claims: 1-46 partially

An isolated DNA molecule comprising a nucleotide sequence substantially similar to SEQ.ID.N.3, wherein the sequence encodes an amino acid sequence similar to SEQ.ID.N.4 which is isolated from a plant and has "5283" activity; an expression cassette; a recombinant vector; a host cell; a transformed plant; a method for obtaining shuffled sequences from SEQ.ID.N.3; a method for selecting compounds interacting with the encoded protein; inhibitors of "5283" activity; a process of identifying compounds having herbicidal activity.

3. Claims: 1-46 partially

An isolated DNA molecule comprising a nucleotide sequence substantially similar to SEQ.ID.N.5, wherein the sequence encodes an amino acid sequence similar to SEQ.ID.N.6 which is isolated from a plant and has "2490" activity; an expression cassette; a recombinant vector; a host cell; a transformed plant; a method for obtaining shuffled sequences from SEQ.ID.N.5; a method for selecting compounds interacting with the encoded protein; inhibitors of "2490" activity; a process of identifying compounds having herbicidal activity.

4. Claims: 1-46 partially

An isolated DNA molecule comprising a nucleotide sequence substantially similar to SEQ.ID.N.7, wherein the sequence encodes an amino acid sequence similar to SEQ.ID.N.8 which is isolated from a plant and has "3963" activity; an expression cassette; a recombinant vector; a host cell; a transformed plant; a method for obtaining shuffled sequences from SEQ.ID.N.7; a method for selecting compounds interacting with the encoded protein; inhibitors of "3963" activity; a process of identifying compounds having herbicidal activity.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

5. Claims: 1-46 partially

An isolated DNA molecule comprising a nucleotide sequence substantially similar to SEQ.ID.N.9, wherein the sequence encodes an amino acid sequence similar to SEQ.ID.N.10 which is isolated from a plant and has "4036" activity; an expression cassette; a recombinant vector; a host cell; a transformed plant; a method for obtaining shuffled sequences from SEQ.ID.N.9; a method for selecting compounds interacting with the encoded protein; inhibitors of "4036" activity; a process of identifying compounds having herbicidal activity.

INTERNATIONAL SEARCH REPORT

Information on patent family members

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